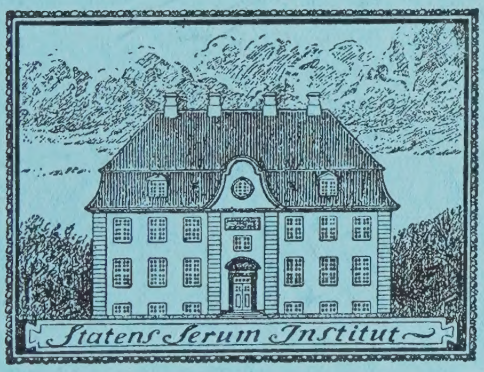
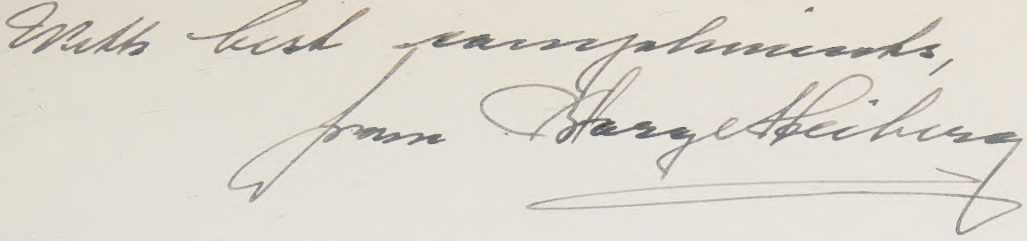
ON THE CLASSIFICATION OF VIBRIO CHOLERÆ AND THE CHOLERA-LIKE VIBRIOS

by

BØRGE HEIBERG



NYT NORDISK FORLAG · ARNOLD BUSCK
KJØBENHAVN MCMXXXV



ON THE CLASSIFICATION OF VIBRIO CHOLERÆ AND THE CHOLERA-LIKE VIBRIOS

Translated from Danish
by
Dr. Gerda Seidelin Schnohr, Copenhagen,
with the assistance of
Dr. G. Gregory Kayne, London.

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KJØBENHAVN MCMXXXV

Denne Afhandling er af det lægevidenskabelige Fakultet antaget til offentlig at forsvares for den medicinske Doktorgrad.

København, den 29. Maj 1935.

RICH. EGE
h. a. dec.

KROHNS BOGTRYKKERI
HARALD JENSENS BOGTRYKKERI

Preface.

The present investigation has been carried out whilst holding an assistantship at the State Serum Institute, Copenhagen.

My most respectful thanks are due to the Director of the Institute, Dr. Th. Madsen, for encouraging me to undertake the work, for his advice and help, for his never failing interest and for the ideal conditions which I have enjoyed for my work.

Only with the kind assistance of the following different bacteriological institutes have these investigations been at all possible: The School of Tropical Medicine, Calcutta; The Central Research Institute, Kasauli; The Public Health Laboratory, Hangchow; The Public Health Laboratory, Shanghai; The Bureau of Quarantine Service, Manila; The Pasteur Institute, Saïgon, The Laboratorium voor Gezondheidzleer, Amsterdam; and the National Collection of Type Cultures, London.

The Rask-Ørsted Foundation helped by making it possible for me to go to Amsterdam, where I was able to study special technical methods under Professor van Loghem.

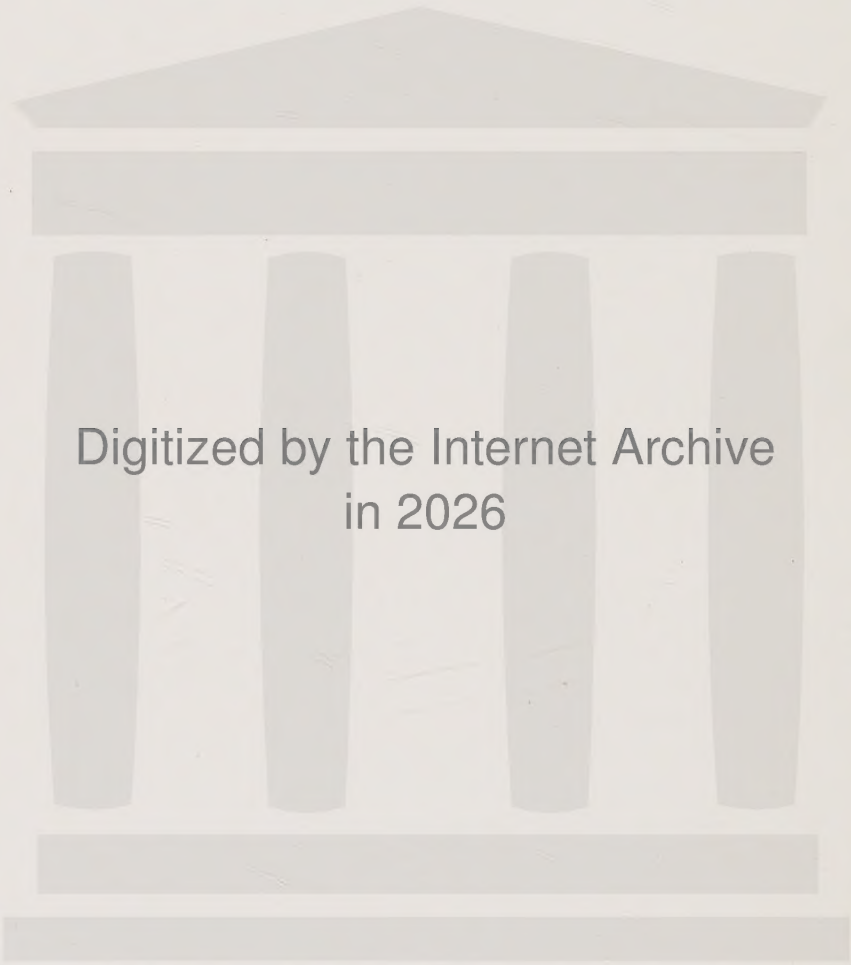
In my daily work and in discussions at the State Serum Institute I had much advice and encouragement from many members of the Staff; particularly has the knowledge and experience of Dr. Martin Kristensen, head of the bacteriological department, and that of Dr. F. Kauffmann been of infinite value to me; these they kindly placed freely at my disposal.

Miss Vibeke Løvgren, Mrs. Ruth Møller and Miss Grete Schiøttz have been of much assistance in carrying out the experiments.

To all these I should like to express my sincerest thanks.

Copenhagen, June 1935.

Børge Heiberg.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Introduction.

IN October 1932 at a meeting of the Cholera Committee appointed by the Office International d'Hygiène Publique, of which Dr. Th. Madsen is a member, the importance was discussed of producing, if possible, an agglutinating standard cholera serum for use in bacteriological diagnosis. This discussion was prompted by the repeated finding at the quarantine station El Tor of vibrios, the true identity of which was doubted by several investigators, in spite of their agglutination with the cholera serum of the quarantine station as well as with other cholera sera. The findings at El Tor will be described in greater detail later.

The Cholera Committee asked Dr. Th. Madsen, the Director of the State Serum Institute at Copenhagen, and Professor Cantacuzène of Bucharest, to have investigations carried out on whether, at the present time, it was possible to produce an agglutinating standard serum for general diagnostic work. Professor Cantacuzène's work had only just begun, when his death took place, shortly after the meeting just mentioned. Dr. Th. Madsen delegated the investigation of the problem to the author of this paper in the New Year of 1933.

It will be easily understood, that the final answer to the question cannot be given in a place so remote from districts where cholera is a common disease, and where the necessary co-operation between bacteriologist and clinician is obtainable; on the other hand, it should be possible to make the preliminary essential investigations, including classification of the vibrios, here. These preliminary investigations are described below. In addition, institutions all over the world have simultaneously been examining several agglutinating standard sera as to their agglutinating power against different vibrios, amongst others a serum prepared at the Danish State Serum Institute in 1932. The results of these examinations were reported at a meeting of the Cholera Committee in May 1934 together with a summary of the work described in this paper.

Chapter I.

PRELIMINARY REMARKS

In reviewing the Gram-negative, rod-shaped bacteria of the intestine numerous well-defined groups are found. By means of biochemical and serological reactions it has been possible to establish a well-defined scheme for the identification of most of these bacteria.

A different state of affairs exists, however, in regard to the Gram-negative, comma-shaped bacteria, known as vibrios. The only one commonly considered pathogenetic to man is the Vibrio Choleræ. But the pathogenicity of numerous vibrios differing from the Vibrio Choleræ in some of their laboratory reactions has either not been determined at all, or insufficiently to allow precise labelling. Slight attempts at defining other groups have been made, for instance, those bearing the names of Vibrio Choleræ A and B; but, on the whole, a more definite classification of vibrios not belonging to the group of Vibrio Choleræ is lacking. And even within the group of vibrios called Vibrio Choleræ by some authors, there are species, the correct diagnosis of which is doubted by other investigators.

It will thus be seen that within the range of laboratory work on vibrios, two big problems are still unsolved: the general classification based on biochemical and serological reactions, and the special investigation of the vibrios forming the link between those definitely belonging to the group Vibrio Choleræ, and those which definitely do not belong to it. Yet another problem, the determination of the pathogenicity of the vibrios, can only be solved by co-operation with clinicians.

In 1883—1884, before serological reactions were used for the classification of bacteria, Robert Koch published the first reports on the vibrios he had found both in the intestine of about 100 patients, who died from cholera, and in the excreta and objects which had been in contact with the excreta of living cholera

patients. As neither Koch himself nor other investigators had found comma-shaped bacteria elsewhere than in connection with patients suffering from cholera, Koch called the bacillus he had found *comma bacillus* meaning *the producer of cholera,* as he considered its shape alone sufficient for identification.

Apart from carrying out elaborate epidemiological examinations, Koch also carefully investigated the comma bacillus in his laboratory with the methods then available. Besides the shape of the bacillus, he also described its motility and the property of causing gelatin liquefaction as characteristic features. Koch emphasized the great importance of looking for comma bacilli in material that had not been connected with cholera in any way; he had himself made several such investigations, and in the detailed report on the comma bacillus he read before a select gathering of scientists in Berlin in 1884, he encouraged others to continue these investigations.

Negative findings in such examinations would clearly be of importance as further proof of the comma bacillus being the cause of cholera. Koch felt that he lacked a basis for his view on the etiology of cholera on two points: he had not succeeded in causing choleralike symptoms by inoculating animals, nor had the bacteria been found in the infected organism outside the intestinal tract. The latter fact was surprising, considering the acute and serious course of the disease and its effect upon the whole organism. In 1884 Koch states that he cannot disprove the hypothesis that the large number of comma bacilli found in the intestine in the course of the disease may be a secondary phenomenon — a sudden incidental multiplication of one of the numerous intestinal bacteria, due to favourable conditions for growth; but at the same time he dissociates himself from this hypothesis, giving amongst others the reason that hitherto comma bacilli had only been found in connection with clinical cases of cholera. Koch's view can be clearly deduced from the following words in his speech at the Cholera Conference mentioned above: »Ich glaube also mit Bestimmtheit aussprechen zu können, dass die Kommabacillen konstante Begleiter des Koleraprozesses sind, und dass sie nirgends anderswo vorkommen.« The first part of his statement was soon confirmed by numerous investigations, while the latter was disproved quite as soon.

Only a month after the Cholera Conference in 1884, Finkler and Prior reported that they had found comma bacilli in the fæces of several cases of »cholera nostras« at Bonn. This had been a small, isolated epidemic — about 30 cases — in which the patients had had cholera-like symptoms. In the fæces of several of them comma bacilli were found, which morphologically could not be distinguished from the comma bacillus of Koch. Basing themselves on the course of the disease and its epidemiological characters, the authors felt they were entitled to exclude the diagnosis of asiatic cholera. Attempts at cultivation failed. In view of these findings, they maintained that it was not possible to make the bacteriological diagnosis by means of the microscope alone.

A few weeks after this first report, Finkler announced that cultivation had been succesful in one case. He had succeeded in growing a comma bacillus, which unlike that described by Koch, produced bodies, which Finkler on account of their morphology termed spores.

Early in 1885 Dencke of Göttingen reported investigations with a bacterium cultivated from cheese, morphologically resembling the comma bacillus. He compared the cultural characters of this bacillus with those of Koch's and Finkler-Prior's vibrios, and he found small differences in their growth on gelatin, potato and agar.

In 1885 Miller in Germany isolated a comma bacillus from the mouth; amongst other features this bacillus differed from Koch's comma bacillus in growing more rapidly on gelatin.

It will be noted that the cultural characters were now beginning to play a rôle in the differential diagnosis of the comma bacilli; but at a time when cholera was a disease which had to be reckoned with in Europe also, it was not felt to be very safe to base the differential diagnosis between the cholera bacillus and other morphologically similar bacteria on the rate of growth on gelatin and similar reactions. In fact the epidemiological factors were also essential in the »bacteriological« diagnosis.

It was, therefore, considered a great step forward, when in 1887 Bujwid of Warsawa and, shortly after, Dunham (1887) of Berlin, published investigations of a test, the cholera-red reaction, which they considered specific for the cholera bacillus. The addition of a small amount of sulphuric or hydrochloric acid to a 24 — hours'

bouillon or peptone — water culture of cholera bacteria produced a distinctly red colour. None of the other comma bacilli hitherto isolated or other bacteria caused this colour. Other investigators soon found that the reaction was due to the simultaneous presence of indole and nitrites, two substances formed by the cholera bacterium from the culture medium (Salkowski (1887)). But Salkowski thought this fact did not prove the specificity of the reaction.

This reaction has a prominent place in the subsequent publications on the comma bacilli. In the blood of birds affected with a disease clinically and pathologico-anatomically resembling cholera, Gamaleia 1888 found a vibrio which he called Vibrio Metchnikowi. It differed from the Vibrio Choleræ by not producing the cholera-red reaction. Neither did a vibrio isolated in 1893 by Wolf from the cervix of a patient suffering from endometritis produce this reaction. Here it is necessary to point out, that both Bujwid and Dunham emphasized the importance of using pure cultures when carrying out the test. Growth of other bacteria might possibly interfere with the appearance of the reaction, as other bacteria may decompose the nitrites, one of the essential factors of the reaction thus missing.

Following the large cholera epidemic in Hamburg in 1892, when it was found that the water from the Elbe was the source of infection, the water was regulary examined for vibrios in subsequent years. In 1893 these examinations disclosed a vibrio which, according to Dunbar's reports, greatly resembled the Cholera vibrio, but he was not able definitely to establish its identity. He did not consider the fact that it produced cholera-red decisive. This proved support for the doubt expressed as to the specificity of this reaction.

In 1894 Brix who had himself found a vibrio in sputum containing pneumococci, stated that 14 different vibrios had been described up to then; but except for their cultural characters and the cholera-red reaction, criteria by which to group them were still lacking. An additional criterion, however, was soon available.

In the course of their experiments on immunity in cholera, Pfeiffer and Issaeff (1894) had observed that vibrios injected intraperitoneally in actively or passively immunized guinea-pigs were soon destroyed. They carried out more detailed examinations of the specificity and mechanism of this phenomenon, and late in 1894 and in

the beginning of 1895 Pfeiffer described the precise technique and experimental basis of the reaction which has since born his name: the Pfeiffer phenomenon.

The reaction is carried out by the simultaneous, intraperitoneal injection in a guinea-pig of cholera immune serum and of the culture to be examined. Every ten minutes a sample of the peritoneal contents is removed with a capillary pipette, and, if the vibrio is a cholera vibrio, microscopic examination of this fluid, best carried out in a hanging drop preparation, shows immediate cessation of motility, and during the following hour, total bacteriolysis of the vibrios. If the vibrios injected belong to another group, they remain entirely unaffected, both as regards motility and the condition of their bodies. As the result of investigating many species, isolated from different material, Pfeiffer concluded that the reaction was specific, and gives it as the only criterion by means of which the laboratory can decide whether or not a vibrio is a cholera vibrio.

Pfeiffer discussed the cause of the phenomenon and, as it was so clearly connected with the living infected organism, he believed that the bactericidal substances were formed by the interaction of the peritoneal endothelium and the injected serum. He felt justified in excluding phagocytosis as the decisive factor.

Although the reaction of Pfeiffer has been succeeded by simpler reactions, it remained for long the decisive test when doubt arose in the diagnosis of cholera, as to whether or not a vibrio was a cholera vibrio. As a practicable diagnostic reaction it was soon replaced by the agglutination test.

The general observations on the agglutination phenomenon made by Gruber and Durham (1896) were carried out on cholera vibrios and typhoid bacilli. In their reports they described both agglutination in hanging drop preparations and in the test tube. In the following years the technique of the agglutination tests was worked out in detail, both as regards obtaining the serum (Madsen and Jørgensen 1902 amongst others) and performing the reaction, thus making it both practicable and reliable.

Kolle and Gotschlich in 1903 published their results of extensive investigations on the specificity of the agglutination test with regard to the vibrios, and the Vibrio Choleræ in particular. They also tried to elucidate the problem whether vibrios other than

the Vibrio Choleræ Koch could produce cholera. Their material consisted partly of vibrios isolated in Egypt under well-known epidemiological conditions, and partly of laboratory strains isolated in different places. The strains were examined as to morphology, Pfeiffer's phenomenon and agglutination.

The authors emphasize the difficulties in the agglutination test due to the age of laboratory strains, as well as to other causes of error, causes which, after 30 years, still lead to mistakes and misinterprepations.

As a result of their work, they felt justified in maintaining that an agglutination test correctly carried out showed unerring specificity between the antigens and antibodies of the Vibrio Choleræ Koch. Further, they were of opinion that the clinical picture of asiatic cholera could only be produced by the vibrio of Koch; the last statement they based on the experience that profuse numbers of vibrios other than the Vibrio Choleræ had never been observed in patients. In those cases in which cholera-like vibrios had been found in cholera patients, they were only few, and most often present together with cholera vibrios.

With the knowledge of serological methods then available, Kolle and Gotschlich's work necessarily impressed as convincing, in view of their careful technique and the consideration of possible sources of error; but one method of examination, introduced a few years later, had not been used, namely, the absorption of antibodies from the sera with different strains.

It was to be expected that the work of Kolle and Gotschlich would secure peace in regard to the question of the bacteriological diagnosis of the Vibrio Choleræ, when the agglutination reaction was being used as the decisive test for the identification of a vibrio; but in 1905—1906 a discussion began, which has continued since. It arose out of the work of F. Gotschlich (1906) working at the quarantine station El Tor at the south point of the Sinai peninsular. He isolated vibrios agglutinable by cholera serum, and therefore considered as cholera vibrios; but later two points caused doubt as to their identity with the Vibrio Choleræ: the epidemiological characters, and the fact that they could produce hæmolysin, the last not having been observed in connection with the Vibrio Choleræ previously.

As early as 1897, at the same quarantine station, a vibrio, which serologically seemed identical with the Vibrio Choleræ, had been isolated from the fæces of a pilgrim, who had shown no clinical symptoms of cholera; but as no cases of cholera had occurred at that time, the head of the quarantine station had not dared to take the responsibility of declaring the vibrio a true cholera vibrio. The repeated finding of the vibrio under similar conditions in 1905 caused a close examination of the isolated vibrios. Kraus and Pribram now (1906) found that these strains produced a hæmolysin, identical with that produced by other cholera-like vibrios. This hæmolysin could not be found in what they called true cholera vibrios, and they therefore drew the conclusion that the vibrios isolated at El Tor in 1905 were not true cholera vibrios. This conclusion also seemed to be borne out by the result of epidemiological investigations.

Since then it has been disputed whether a hæmolytic vibrio could cause cholera. On the one hand it is maintained (Kolle, Prigge, Pfeiffer, E. Gotschlich etc.) that from the point of view of diagnosis the power of producing hæmolysin cannot be used in preference to the agglutination reaction and the Pfeiffer phenomenon, and that this property may be found in vibrios isolated from clinically proved cases of cholera. On the other hand it is stated (Kraus, Greig, v. Loghem etc.) that almost all the cholera-like vibrios possess the power of producing hæmolysin, and that freshly isolated vibrios from clinically proved cholera have never been found hæmolytic.

As is so often the case in scientific discussions errors in experimental technique play a rôle in causing the disagreement. Kraus had already seen these difficulties. Amongst other factors, the species of animal from which the erytrocytes are taken, is of importance, and also whether the examination is carried out on a solid or a liquid medium.

The controversy subsided for several years, although agreement was not reached, and the question again became topical, when in 1930/1931 vibrios agglutinable by cholera serum were again isolated at El Tor, although cholera had not been diagnosed for a long time in the areas from which the would-be carriers came. Unfortunately the vibrios found were not examined immediately as to their hæmo-

lytic power. Not until they had been inoculated several times were they examined in this manner, when they exhibited strong hæmolysis in the cultures. Discussion again arose and doubt expressed as to whether El Tor should have been declared cholera-stricken.

The solution of the problem of the identity of the El Tor strains has also been attempted by means of special serological examinations. In 1907 Neufeld and Händel examined the complement-fixation tests of the strains, but even by this method they were unable to distinguish the El Tor strains from true cholera strains. In 1921 Douglas tried to distinguish between them by absorption as well as by agglutination experiments; he was also unsuccesful. But both Neufeld and Händel, and Douglas used an imperfect technique, so that their examinations did not give the final proof of the serological identity of these strains.

The different attempts, which have in the meantime been made to name different groups of vibrios on the basis of serological reactions, have hitherto not led to any practical mode of classification.

The question has been examined from different points of view. The possibility that the strains agglutinated by cholera immune serum included several types has been considered as well as that some of the strains which do not agglutinate might produce clinical and epidemiological cholera. The third possibility was that amongst the vibrios there might be pathogenetic microbes, causing clinical diseases different to cholera.

The first possibility, the existence of several types within the group of vibrios agglutinable by cholera serum, was already discussed by Kolle and Gotschlich in connection with their work in 1903. They believed, that there was only one serological type of the Vibrio Choleræ Koch. In 1921 Douglas worked on this problem and came to the same conclusion. And, as has already been mentioned, other investigators had examined the serological relations between the Vibrio Choleræ and the El Tor strains, and had also reached the same conclusions.

In regard to the second possibility — whether vibrios that are inagglutinable by cholera serum are able to produce clinical cholera — Kabeshima in 1913 thought he had proved the existence of two serologically different vibrios that might produce cholera: one, later called *type original,* was identical with the ordinary cholera

vibrio and had been isolated from a cholera epidemic; whilst the other, later called *type varié,* was inagglutinable by cholera serum and had been isolated from sporadic cases of cholera. When, however, it is a question of deciding whether a new vibrio is the cause of cholera, it is essential, that it should have been isolated under conditions which do not allow of any doubt as to the clinical diagnosis. Further, Kabeshima in his laboratory considered that he could cause a return of the characteristics of the new vibrio to those of the classical one. In 1923 Nobechi stated that he had found a third vibrio as the cause of cholera. It was supposed to form the link between the two vibrios of Kabeshima. These 3 strains are included in the material investigated by the author, and in the chapter on their serological reactions their characteristics will be more fully discussed.

In Egypt Mackie and Storer (1917) isolated two different vibrios in a small epidemic of diarrhoea; they called them vibrio Paracholeræ A and B. They were both inagglutinable by cholera serum. Finally a vibrio corresponding to Kabeshima's *type varié* has been isolated at Shanghai from numerous clinical cases of cholera. With the exception of these two investigations, and the one previously mentioned, by Finkler in 1883, cholera-like epidemics, in which vibrios other than the true cholera vibrio were considered to be the cause of the disease, have not been described. It is true, that Greig in 1917 reported investigations on cholera-like vibrios isolated from cholera patients, but he does not discuss the problem of their pathogenecity. In some of the cases he had isolated agglutinable vibrios at the same time. In an attempt to divide 75 vibrios inagglutinable by cholera serum into groups, he found that 62 could be placed within 9 groups, whilst 13 had still to be left out.

The third reason which might make a more detailed grouping of the vibrios desirable is that some vibrios could possibly cause clinical pictures different from that of cholera. Some of the cases of Mackie and Storer belong to this group, but so far they have not been methodically examined. An attempt at methodical examination was made in Russia in 1932 (Kwashina), but as the work was confined to the laboratory, and neither the clinical nor the epidemiological features were taken into consideration, it is of no significance in this connection.

Weil and Felix, who were the first to investigate the importance of different thermostabile and thermolabile antigens in regard to the grouping of pathogenetic, Gram-negative rods, also considered the Vibrio Choleræ, in order to trace similar antigenic factors, and found that the Vibrio Choleræ produced thermostabile antigen only. More recent examinations of these factors have shown varying results. Brutsaert in 1924 confirmed the findings of Weil and Felix, whilst Balteanu, Gohar, Abdoosh and later Gardner maintained that they had found thermolabile as well as thermostabile antigen. Gohar and Abdoosh only have dealt with the importance of the thermolabile and thermostabile antigens in relation to the grouping of the vibrios and they came to different conclusions.

The purpose of the present investigation has been, first of all, to find and emphasize both the general characteristics and the individual characteristics of a large vibrio material. On the basis of these findings an attempt has been made to establish a classification each group in which contains bacteriologically closely related or, more often, identical vibrios.

The author also gives his conclusions in regard to the possibility of producing an agglutinating standard serum at the present time.

Chapter II.

THE MATERIAL

The material investigated includes about 400 strains of bacteria, sent to the State Serum Institute, Copenhagen, as vibrios. The strains were indexed at random as continuous numbers from 1 to 412. All the 412 have, however, not been examined, as 24 of the strains were found on arrival to be not alive, or were lost during the progress of the work. In addition, 4 strains were used for very special examinations only. The strains were sent to the Serum Institute with the designation »agglutinable« cholera vibrios or as »inagglutinable« vibrios, i. e. inagglutinable by cholera serum; both these groups were isolated from clinical cases of cholera. There were also investigated vibrios isolated at El Tor from patients without clinical cholera, water vibrios, and a few others. In a few cases the same strains were received from 2 different laboratories. Both samples were generally included in the investigations, in order to control the accuracy of the findings. In a few cases, moreover, several cultures were isolated from the same patient.

Most of the strains were sent from institutes in cholera districts to the Serum Institute on request for vibrios agglutinable with the cholera test sera of the institutes, and for strains inagglutinable with these sera. Included also are some strains sent to the general bacteria collection of the Institute.

The strains have been kept as stab cultures in meat infusion agar — ½ % meat infusion, 1 % Riedel peptone, ½ % sodium chloride, — at room temperature. Subcultures were made every 3rd month; immediately after the inoculation the cultures were incubated at 37° C. for 20 hours.

Below is given a list of the vibrios. The first column shows the register number of the strain in the present investigation, the next the

designation with which the strain had been sent to the Serum Institute; details of the time of its inclusion in the collection of the Serum Institute, the laboratory from which it has been received, the time of its isolation and from where it has been isolated then follow. Where no data have been put down, information is lacking.

List of material, see p. 22—45.

LIST OF

No.	Designation	Received 19	Received from
1.	V. chol. Manila 1	32	Eastern Bur. Health Organ.
2.	- — — 2	—	—
3.	- — — 3	—	—
4.	- — — 4	—	—
5.	- — — 5	—	—
6.	- — — 6	—	—
7.	- — — 480	—	—
8.	- — — 30/529	—	—
9.	Siam B-1	—	—
10.	— B-2	—	—
11.	— B-3	—	—
12.	Jalandoni	—	—
13.	Masbate-215832	—	—
14.	Bulacan-219617	—	—
15.	Bandoeng 1	—	—
16.	— B	—	—
17.	Banuelos	—	—
18.	Dumaguete 009/10	—	—
19.	— 009/9	—	—
20.	069/1	—	—
21.	Pasig 027/9	—	—
22.	V. chol. 1154	—	Saigon Pasteur Inst.
23.	- — 1155	—	—
24.	Dumaguete 009/3	—	Eastern Bur. Health Organ.
25.	V. chol. K 1473	—	Saigon Pasteur Inst.
26.	Manila Ha-10	—	Eastern Bur. Health Organ.
27.	— Ha-11	—	—
28.	— Ha-19	—	—
29.	Bulacan 215530/1	—	—
30.	— 215530/2	—	—
31.	J. Arellano	—	—
32.	Chol. Kasauli 1485	—	Centr. Res. Inst. Kasauli
33.	— — 173	—	—
34.	— — 3222/1	—	—

MATERIAL

Time of isolation	Isolated from	Remarks
1924	Case of cholera	
1930	—	
1930	—	
1930	—	
1930	—	
1930	—	
1930	—	
1930	—	
1930	—	
1930	—	
1930	—	
1931	—	
1924	—	
1924	—	
1924	—	
1930	—	
1930	—	
1930	—	

No.	Designation	Received 19	Received from
35.	Chol. Kasauli 77	32	Centr. Res. Inst. Kasauli
36.	— — 3214/4	—	—
37.	— — 1410/1	—	—
38.	— — 1416/1	—	—
39.	— — 3205/2	—	—
40.	— — 11	—	—
41.	— — 1486/2	—	—
42.	Mrs. Grandi, V. Chol.	28	Kings Inst. Madras
43.	Bukmani, V. Chol.	—	—
44.	Ny , —	—	—
45.	Msv., V. Chol.	—	—
46.	V. Chol. Abdel Kader	—	—
47.	Kommavibr. Bed Nr. 10	—	Calcutta
48.	V. Chol. Gabetz	26	
49.	- — Pottevin	—	
50.	- — 7932	20	Polen
51.	- — 7921	—	—
52.	- — 7374	—	—
53.	- — 49	—	—
54.	- — J. P.	19	Inst. Pasteur, Paris
55.	- — 11080	18	Inst. f. Infektionskrankh.
56.	- — 10929	—	—
57.	- — Sjøgreen	—	Stockholm
58.	- — Nybäck	—	—
59.	Nasik	21	Inst. f. alm. Patol., Kbhvn.
60.	Spir. Metchnikow	—	—
61.	— Metchnikow-Bordet	26	
62.	Chol. Jenkins NC 1633	26	Nat. Coll. Type Cult., London
63.	— Bombay NC 29		—
64.	— Kedah NC 22	26	—
65.	— Pfeifferi NC 27		—
66.	— Freemann NC 14		—
67.	— Singapore NC 21		—
68.	Parachol. B-Mackie NC 559	33	—
69.	— Martin NC 50	—	—
70.	— A-Mackie NC 558	—	—

Time of isolation	Isolated from	Remarks
	Case of cholera	
	—	
	—	
1921	—	
	—	Discarded. No growth on subculture
1895	—	
	Case of Diarrhoea	
	Case of Diarrhoea	

No.	Designation		Received 19	Received from
71.	El Tor 4	NC 307	33	Nat. Coll. Type Cult., London
72.	Choleralike	NC 3651	—	—
73.	—	NC 3650	—	—
74.	V. Cholerae	NC 23	—	—
75.	Choleralike	NC 3636	—	—
76.	V. Cholerae	NC 3638	—	—
77.	Choleralike	NC 418	—	—
78.	El Tor	NC 1548		—
79.	Choleralike	NC 3646	—	—
80.	—	NC 717	—	—
81.	El Tor D_{64}	NC 3658	—	—
82.	Choleralike	NC 689	—	—
83.	—	NC 2084	—	—
84.	—	NC 686	—	—
85.	—	NC 3648	—	—
86.	—	NC 3647		—
87.	—	NC 3634	—	—
88.	—	NC 3654	—	—
89.	El Tor	NC 2890	—	—
90.	Cholerae	NC 3639	—	—
91.	—	NC 3642	—	—
92.	Choleralike	NC 3648	—	—
93.	—	NC 3649	—	—
94.	El Tor D_{47}	NC 3660	—	—
95.	Chol. Brit. Ind.	228	—	Lab. v. Gezondhl., Amsterdam
96.	— —	262	—	—
97.	— —	264	—	—
98.	— —	266	—	—
99.	— —	268	—	—
100.	El Tor A		—	—
101.	— D_6		—	—
102.	— D_{20}		—	—
103.	— D_{47}		—	—
104.	— D_{49}		—	—
105.	— D_{67}		—	—
106.	— D_{80}		—	—

Time of isolation	Isolated from	Remarks
1931		
1929		
1920		
1929		
1932		
1917		Discarded. Overgrown by bac. subtilis
1923		
1929		
1917		
1930		
1920		
1917		
1931	Water	
1931		
1932		
1931		
1905		
1932		
1932		
1931	Water	Not examined; identical with No. 85
1931		
1931		
1905		
1930—31		
1930—31		
1930—31		
1930—31		
1930—31		
1930—31		

No.	Designation	Received 19	Received from
107.	Chol. August 31.	33	Centr. Res. Inst. Kasauli
108.	— 7812	—	—
109.	— (?) 79	—	—
110.	— 51	—	School Trop. Med., Calcutta
111.	— 53	—	—
112.	— 57	—	—
113.	— 58	—	—
114.	— 63	—	—
115.	— 500	—	—
116.	— 588	—	—
117.	— 618	—	—
118.	— 836	—	—
119.	— 1168/2	—	—
120.	— 1169	—	—
121.	— 1233	—	—
122.	— 1246	—	—
123.	— 1250		—
124.	— 1252	—	—
125.	— 1257	—	—
126.	— 1260	—	—
127.	— 1273/2	—	—
128.	— 1274	—	—
129.	— 1279	—	—
130.	— 1290	—	—
131.	— 1307	—	—
132.	— 1319	—	—
133.	— 1370	—	—
134.	— 1401/6	—	—
135.	— 1405/6	—	—
136.	— 1406/1	—	—
137.	— 1410/1	—	—
138.	— 1410/3	—	—
139.	— 1418	—	—
140.	— 1470	—	—
141.	— 1480	—	—
142.	— 1484		—

Time of isolation	Isolated from	Remarks
1931		
7/3—29	Case of cholera	
		Discarded. Not viable when received.
8/3—29	—	
8/3—29	—	
9/3—29	—	
2/6—29	—	
12/7—29	—	
19/7—29	—	
23/9—29	—	
7/1—31	—	
6/1—31	—	
24/2—31	—	
3/3—31	—	
5/3—31	—	
5/3—31	—	
7/3—31	—	
10/3—31	—	
13/3—31	—	
14/3—31	—	
2/4—31	—	
14/4—31	—	
25/4—31	—	
22/5—31	—	
4/11—31	—	
5/1—32	—	
11/1—32	—	
12/1—32	—	
22/1—32	—	
22/1—32	—	
4/2—32	—	
17/5—32	—	
27/5—32	—	Discarded. Not viable when received.
31/5—32	—	

No.	Designation	Received 19	Received from
143.	Chol. 1486/2	33	School Trop. Med. Calcutta
144.	— 1528	—	—
145.	— 1537	—	—
146.	— 1543	—	—
147.	— 1544	—	—
148.	— 1549	—	—
149.	— 1554	—	—
150.	— 1609/1	—	—
151.	— 1609/2	—	—
152.	— 1610	—	—
153.	— 1611	—	—
154.	— 1614	—	—
155.	— 1615	—	—
156.	— 1618	—	—
157.	— 1619	—	—
158.	— 1621	—	—
159.	— 1622	—	—
160.	— 1623	—	—
161.	— 1624	—	—
162.	— 1628	—	—
163.	— 1630	—	—
164.	— 1632	—	—
165.	— 1633	—	—
166.	— 1634	—	—
167.	— 1637	—	—
168.	— 1638	—	—
169.	— 1647	—	—
170.	— 1648	—	—
171.	— 1649	—	—
172.	— 1651/1	—	—
173.	— 1651/2	—	—
174.	— 1653	—	—
175.	— 1654	—	—
176.	— 1655	—	—
177.	— 1656	—	—
178.	— 1658	—	—

Time of isolation	Isolated from	Remarks
9/6—32	Case of cholera	
1/9—32	—	
20/11—32	—	Discarded. Not viable when received.
25/11—32	—	
25/11—32	—	
3/12—32	—	
7/12—32	—	
13/1—33	—	
13/1—33	—	
14/1—33	—	
14/1—33	—	
17/1—33	—	
17/1—33	—	
21/1—33	—	
21/1—33		
23/1—33	—	
24/1—33	—	
24/1—33	—	
25/1—33	—	
1/2—33	—	
2/2—33	—	
3/2—33	—	
4/2—33	—	
4/2—33	—	
8/2—33	—	
9/2—33	—	
17/2—33	—	
17/2—33	—	
17/2—33	—	
20/2—33	—	
20/2—33	—	
21/2—33	—	
21/2—33	—	
22/2—33	—	
22/2—33	—	
22/2—33	—	

No.	Designation	Received 19	Received from
179.	Chol. 1659	33	School Trop. Med., Calcutta
180.	— 1661	—	—
181.	— 1662	—	—
182.	— 1665	—	—
183.	— 1666	—	—
184.	— 1667	—	—
185.	— 1668	—	—
186.	— 1670	—	—
187.	— 1673	—	—
188.	— 1674	—	—
189.	— 1675	—	—
190.	— 1676	—	—
191.	— 1677	—	—
192.	— 1678	—	—
193.	— 1682	—	—
194.	— 1683	—	—
195.	— 1685	—	—
196.	— 1688	—	—
197.	1604	—	—
198.	— 1606/1	—	—
199.	— 1606/2	—	—
200.	— 1687	—	—
201.	— 1690	—	—
202.	— 1691	—	—
203.	— 1693	—	—
204.	— 1695	—	—
205.	— 1703	—	—
206.	— 1707	—	—
207.	— 1708	—	—
208.	— 1709	—	—
209.	— 1711	—	—
210.	— 1712	—	—
211.	— 1716	—	—
212.	— 1719 A	—	—
213.	— 1720	—	—
214.	— T	—	—

Time of isolation	Isolated from	Remarks
22/2—33	Case of cholera	
24/2—33	—	
24/2—33	—	
23/2—33	—	Discarded. No growth on subculture
27/2—33	—	
27/2—33	—	
27/2—33	—	
28/2—33	—	
2/3—33	—	
3/3—33	—	
3/3—33	—	
3/3—33	—	
3/3—33	—	
4/3—33	—	
4/3—33	—	
6/3—33	—	
6/3—33	—	
8/3—33	—	
9/1—33	—	
11/1—33	—	
11/1—33	—	
8/3—33	—	
9/3—33	—	
9/3—33	—	
9/3—33	—	
10/3—33	—	
15/3—33	—	Discarded. Unpurified by swarming rods.
16/3—33	—	
16/3—33	—	
20/3—33	—	
17/3—33	—	
18/3—33	—	
20/3—33	—	
22/3—33	—	
22/3—33	—	
1928	—	

No.	Designation	Received 19	Received from
215.	Chol. Shillong X	33	School Trop. Med. Calcutta
216.	— — N	—	—
217.	— Sh. 7912	—	—
218.	— Brit. Ind. 80	—	Lab. v. Gezondhl., Amsterdam.
219.	— — 82	—	—
220.	— — 83	—	—
221.	— — 85	—	—
222.	— — 86	—	—
223.	— — 89	—	—
224.	— — 94	—	—
225.	— — 254	—	—
226.	— — 259	—	—
227.	— — 260	—	—
228.	— — 261	—	—
229.	— — 263	—	—
230.	— — 270	—	—
231.	— — 271	—	—
232.	— — 272	—	—
233.	— — 273	—	—
234.	— — 274	—	—
235.	— — 275	—	—
236.	— — 276	—	—
237.	— — 277	—	—
238.	— — 279	—	—
239.	— — 294	—	—
240.	— — 295	—	—
241.	— — 296	—	—
242.	— Ton Akio		—
243.	— Bagdad I		—
244.	— Bussian		
245.	— Bagdad I Cairo	—	—
246.	— — II —	—	—
247.	— Cairo II		—
248.	— — III	—	—
249.	— — VI		—
250.	— Boelan		—

Time of isolation	Isolated from	Remarks
1932		
1932		
1932		

No.	Designation	Received 19	Received from
251.	Chol. 45	33	Lab. v. Gezondh. l., Amsterdam
252.	— D_{16}	—	—
253.	— Deli 193	—	—
254.	Vibr. Dunbar	—	—
255.	D_{70}	—	—
256.	Chol. Freemann	—	—
257.	— Juffe(?) S Nr. I	—	—
258.	Mekka-Medow	—	—
259.	V. Metchnikow	—	—
260.	Chol. Pev 2	—	—
261.	— — 3	—	—
262.	— — 4	—	—
263.	— — 5	—	—
264.	— — 7	—	—
265.	— — 11	—	—
266.	— Saoda		
267.	El Tor D	—	—
268.	V. Cholerae 7	—	Publ. Health Lab. Nanking
269.	— ?	—	—
270.	— 8	—	—
271.	— 59	—	—
272.	— 1931	—	—
273.	— 5	—	—
274.	— ?	—	—
275.	— 1930	—	—
276.	— 29	—	—
277.	— 11	—	—
278.	— 9	—	—
279.	Vibrio Greg. Cent.	—	Bur. Quarant. Serv. Manila
280.	— —	—	—
281.	— —	—	—
282.	Non-agglutin. Vibr. I	—	—
283.	— — III	—	—
284.	Choleralike Vibrio 5	—	—
285.	— — 19	—	—
286.	Non-agglutin. Vibr. III	—	—

Time of isolation	Isolated from	Remarks
1893/95?	Water of Elbe	
		Discarded. Unpurified
1914		
1932		
1932		
1932		Discarded
1932		
		Discarded. Not viable when received
	—	— —
	—	— —
1930		
1932		
1932		Discarded. No growth on subculture
July 32	Case of cholera	
—	—	
—	—	
1925		
1925		
1930		
1924		
1925		

No.	Designation	Received 19	Received from
287.	V. Chol. Greg. Cent.	33	Bur. Quarant. Serv. Manila
288.	Non-agglutin. Vibr. IV	—	—
289.	V. Chol. Type original		
290.	— — interméd.		
291.	— — varié		
292.	Non-agglutin. Vibrio 1411	—	School Trop. Med., Calcutta
293.	— 1347/1	—	—
294.	— 1524	—	—
295.	— 1502	—	—
296.	— 1434/1	—	—
297.	— 1471	—	—
298.	— 1533	—	—
299.	— 1651/2	—	—
300.	— 1622	—	—
301.	— 1457/5	—	—
302.	— 1335/1	—	—
303.	— 1339/7	—	—
304.	— 1447	—	—
305.	— 1355/1	—	—
306.	— 1340/2	—	—
307.	— 1497	—	—
308.	— 1644	—	—
309.	— 1521	—	—
310.	— 1492	—	—
311.	— 1458	—	—
312.	— 1612	—	—
313.	— 1494	—	—
314.	— 1514	—	—
315.	— 1613	—	—
316.	— 1493	—	—
317.	— 1495	—	—
318.	— 1414	—	—
319.	— 1645	—	—
320.	— 1679	—	—
321.	— 1481	—	—
322.	— 1686	—	—

Time of isolation	Isolated from	Remarks
July 32	—	
23/1—32	Case of cholera	
12/9—31		
25/8—32		
30/7—32		
8/4—32		
8/5—32		
7/9—32	Case of cholera	
20/2—33	—	
24/1—33	—	
4/5—32	—	
31/8—31	—	
28/8—31	—	
30/4—32	—	
29/8—31	—	
30/7—32	—	
15/2—33	—	
24/8—32	—	
28/6—32	—	
5/5—32		
16/1—33		
28/6—32	—	
24/8—32	—	
6/1—33	—	Discarded. Lost by mistake
28/6—32	—	
28/6—32	—	
29/1—32	—	
15/2—33	—	Discarded. Lost by mistake
4/3—33	—	
28/5—32	—	
8/3—33	—	

No.	Designation		Received 19	Received from
323.	Non-agglutin. Vibrio	1503	33	School Trop. Med., Calcutta
324.	—	1473	—	—
325.	—	1513	—	—
326.	—	1664	—	—
327.	—	1515	—	—
328.	—	1512	—	—
329.	—	1646	—	—
330.	—	1452	—	—
331.	—	1491	—	—
332.	—	1695	—	—
333.	—	1483/1	—	—
334.	—	1453	—	—
335.	—	817	—	—
336.	—	1223/1	—	—
337.	—	1496	—	—
338.	—	1218	—	—
339.	—	615	—	—
340.	—	1229	—	—
341.	—	840	—	—
342.	—	1229	—	—
343.	Unknown		—	—
344.	Non-agglutin.	633		—
345.	—	1635	—	—
346.	—	827	—	—
347.	—	1094 E	—	—
348.	—	1478	—	—
349.	—	1534	—	—
350.	—	1476	—	—
351.	—	1251	—	—
352.	—	1321/2	—	—
353.	—	1351/1	—	—
354.	—	1449	—	—
355.	—	1097 B	—	—
356.	—	1321/3	—	—
357.	—	1482		
358.	—	1523	—	—

Time of isolation	Isolated from	Remarks
30/7—32	Case of cholera	
19/5—32	—	
24/8—32	—	
24/2—33	—	Discarded. Lost by mistake
24/8—32	—	
24/8—32	—	
6/2—33	—	
1/5—32	—	
28/6—32	—	
10/3—33	—	
28/5—32	—	
1/5—32	—	
13/9—29	—	
10/2—31	—	
28/6—32	—	
9/2—31	—	
18/7—29	—	
11/2—31	—	
25/9—29	—	
13/2—31	—	
		Lost
23/7—29	—	
6/2—33	—	
17/9—29	—	
15/5—30	—	
19/5—32	—	
8/9—32	—	
22/5—32	—	
5/3—31	—	
23/6—31	—	
16/9—31	—	
1/5—32	—	
15/5—30	—	
23/6—31	—	
28/5—32	—	
24/8—32	—	Discarded. No growth on subculture

No.	Designation	Received 19	Received from
359.	Cholera 1455	33	Hongchow, Kina
360.	— 3455	—	—
361.	— 3464	—	—
362.	— 4421	—	—
363.	— 2993	—	—
364.	— 1357	—	—
365.	— 4364	—	—
366.	— 3475		
367.	— 1471	—	—
368.	— K 1473	—	Prof. Cantacuzène, Bucarest
369.	— Pottevin I	—	—
370.	Vibr. Nr. 1, Shanghai	—	Dr. Yang, London (Nanking)
371.	— 2, —	—	—
372.	— 3, —	—	—
373.	— 4, —	—	—
374.	— 5, —	—	—
375.	— 6, —	—	—
376.	— 7, —	—	—
377.	8, —	—	—
378.	— 9, —	—	—
379.	— 10, —	—	—
380.	V. Chol. 56 Nanking	—	—
381.	— 77 —	—	—
382.	— 121 —	—	—
383.	— 123 —	—	—
384.	— 124 —	—	—
385.	— 125 —	—	—
386.	— 126 —	—	—
387.	— 127 —	—	—
388.	Water Vibr. 101	—	—
389.	— 110	—	—
390.	V. Chol. 1		Publ. Health Lab., Shanghai
391.	— 2	—	—
392.	— 3	—	—
393.	Cholera-like A_1	—	—
394.	— A_2	—	—

Time of isolation	Isolated from	Remarks
		Discarded. No growth on subculture
		Discarded. No growth on subculture
1933		
1933		
1933		
1933		
1933		
1933		
1933		
1933		
1933		
1933		
1932		
1932		
1932		
1932		
1932		
1932		
1932		
1932		
1932		
1932		
1932		

No.	Designation	Received 19	Received from
395.	Water Vibr. B_1	33	Publ. Health Lab., Shanghai
396.	— B_2	—	—
397.	Nanking 8/31	—	Nat. Health Adm., Nanking
398.	— 18/31	—	—
399.	— 11/32	—	—
400.	— 62/32	—	—
401.	— 98/32	—	—
402.	— 64/32	—	—
403.	Shanghai 18/31	—	—
404.	— 20/32	—	—
405.	— 30/32	—	—
406.	Hongchow 1453	—	—
407.	Inagglutin. V. 3/32	—	—
408.	— V. 96/32	—	—
406.	Hongchow 1453	34	Inst. Pasteur, Saigon
410.	— 1155	—	—
411.	— K. 1473	—	—
412.	Paracholer. B. Mackie	—	Nat. Coll. Type Cult., London

Abbreviation: N. C. = National Collection of Type Cultures, London.

Time of isolation	Isolated from	Remarks
		Discarded. No growth on subculture
	—	—
1931		
1931		
1932		
1932		
1932		
1932		
1931		
1932		
1932		
		Discarded. Not viable when received
1932		
1932		
1930		
1930		
1931		
1916		

Chapter III.

MORPHOLOGICAL AND CULTURAL CHARACTERS

The group of bacteria dealt with in the present work is limited principally by the morphology, the comma shape*) — observed when recently isolated — being the universal characteristic of the strains examined. Further, they are highly motile, Gram-negative, and grow profusely in aerobic culture on an alkaline medium at 37 ° C..

As the curve of the bacterial body forms a small part of a spiral with coils wide apart — a fact which becomes more apparent in older cultures, in which the individual elements may lie end to end, forming S-forms or longer spirals — some authors (Flügge 1886) have considered the vibrios as belonging to the group of spirilla; but as at any rate all the comma shaped bacteria observed by direct microscopical examination of fæces from cholera patients and of recently isolated young cultures, have shown the characteristic morphological appearence of comma shaped elements, it seems justifiable to define a special group for these bacteria and the bacteria morphologically identical and culturally showing relationship.

Apart from the vibrios growing best at 37° C., there are also vibrios which grow better at 20—22° C. and die after only 24 hours at 37 ° C.. These strains, of special interest in fish-pathology (Bergmann, Schäperclaus, Brun and Heiberg), are outside the scope of the present investigations.

In addition to the shape, the motility is also characteristic. Robert Koch clearly observed this; in his characterisation of the Vibrio Choleræ he compared the microscopical appearance in a hanging drop with that of a swarm of midges.

The motility of the cholera vibrio and many other vibrios is associated with the presence of a single terminal flagellum. Doubt has

*) the name comma shape refers only to the curve of the bacterium. The bacterial body does not show the variation in breadth characteristic of a comma.

been expressed as to whether the cholera vibrios possessed more than one flagellum, several authors, especially before the time of serological examinations, having described multiple flagella; but since serological reactions have formed the basis of bacteriological diagnosis, nearly all authors have agreed that the Vibrio Choleræ is monoflagellate. On the other hand, there are several descriptions of cholera-like vibrios with multiple, terminal flagella; thus Zettnow in Kolle and Gotschlich's paper of 1903 describes 7 strains, some of which had 4—8 terminal flagella, lying amongst other vibrios with fewer flagella. The same paper deals with 13 cholera-like strains which, when specially stained for the demonstration of flagella, could not be distinguished from the true cholera vibrio.

Lack of motility or absence of flagella in recently isolated strains has not been described in literature. Nobechi refers to the lack of these characteristics in 2 museum strains, in which it was impossible either by numerous quickly repeated subinoculations or by animal passage to produce motile elements; this finding shows what might be expected that motility may be absent in laboratory strains.

In the present material, the motility of more than 100 strains, examined from this point of view, was, on the whole, not as active as that seen in recently isolated vibrios. For this reason investigations of motility have not been carried out systematically, and examination of flagella has also been considered as being without interest here; it is possible that such an examination would have revealed cholera-like strains with more than one flagellum, but as it was not possible to exclude the presence of a single flagellum or total absence of flagella as a condition developing in the laboratory, this examination seemed of minor interest in the present attempt to establish a classification.

A remarkable phenomenon, observed in several strains, must be mentioned; whilst the motility of 6—16 and 24 hours' cultures in bouillon (pH 7.6) and of 20-hours' cultures in peptone-water (pH 8—8.4) was very slight, it was much more active in agar surface cultures emulsified in normal saline.

As the definition of the material was thus preferably based on the shape of the bacteria, every one of the strains has been examined in preparations stained by Gram's method.

According to statements in the literature (Kolle and Prigge

1928), the characteristic shape generally disappears in cultures kept in a laboratory for many years, the bacteria becoming rod shaped if inoculation is performed from agar to agar, without the intervention of animal passages. This phenomenon has not been common in the examinations of the present strains.

For the preparations, 20 to 24 hours' agar surface cultures, incubated at 37° C., have been used. The culture was emulsified in 0.9 per cent. sodium chloride. The preparations have been stained by Gram's method. All the strains examined were without exception decolourized by absolute alcohol, and stained bright red in one minute by carbolfuchsin in a dilution of 1 to 10.

Table 1.

Only or mainly comma-shaped elements	333	cultures
Each single element longer, slimmer reminding of a spirillum	17	—
Mainly rod-shaped with few comma-shaped elements	11	—
Rod-shaped elements only	23	—

The bacteria classified according to their appearance in Gram preparations.

The result of these investigation is seen in Table I. By far the greater part — 361 out of 384 strains — appeared to contain comma-shaped elements. In 17 of the strains spirilla-shaped elements were found along with those comma-shaped.

23 strains showed rod-shaped elements only, but some of these strains at any rate have never been vibrios, and were more probably some form of B. fæcalis alcaligenes (Baerthlein (1912)). One strain turned out to be B. coli. These 23 strains have in spite of their rod shape been included in the investigation, as they were sent to the Serum Institute under the name of vibrio.

As regards cultural characters, the strains have not been examined systematically to note differences which might help to classify the vibrios, partly because the former had already unsuccesfully been investigated with the same object in the first years after the discovery of the vibrios, and partly because many bacteria change these characters when kept for long in the laboratory; differences or similarities in various strains are by no means necessarily constant features.

As a part of the other investigations the strains were cultivated in bouillon, peptone-water and on agar, so that strains showing special and unusual type of growth were fully observed.

The following may be called the normal type of growth after 20—24 hours' incubation at 37 ° C.: in veal broth and peptone-water, uniform growth with a tendency towards pellicle formation and development of a loose deposit. In veal broth — pH 7.6 — the uniform growth is generally denser than in the peptone-water (1 % Riedel or Parke Davis peptone with pH 8—8.4). On agar, the growth consists of sharply defined, slightly domed, circular, soft colonies, 3—5 mm in diameter, translucent, and tinged the colour of the agar. Several of the strains moreover produced colonies that were smaller, dense, more domed, whitish and not translucent. Stab cultures in extract agar developed growth both on the surface and along the needle track.

In addition to the fermentation reactions the viability of the cholera vibrios in the fermented and non-fermented media was also examined. From cultures in peptone-water, to which different carbohydrates and alcohols had been added, and the pH of which was 8—8.4 at the beginning of the experiment, subcultures were made in peptone-water at different intervals after the inoculation. In every tube that did not show acid formation (the indicator being bromthymol-blue) living bacteria were still found after 18 days' incubation. Cultures older than this were not examined. In the tubes in which acid formation occurred in the first 24 hours, no living bacteria were found after 7 days; several of the cultures were already sterile at 24 hours. These phenomena indicate the sensitiveness of the cholera vibrio to an acidity greater than 5.5, the pH of the tubes that fermented within the first 24 hours being about 5—5.5.

Rough forms. In bouillon or peptone agar some of the strains were found to form a flocculent deposit, similar to cultures of streptococci. This probably means that these strains are rough, but by spreading on agar the colonies of many of these strains do not differ from the usual smooth type. In a few cases, however, the colonies showed quite a different appearance, being dry, wrinkled, ring shaped, and only a few mm in diameter.

I have not described the different relations of these colonies in details, as this is to a certain extent outside the limits of this

paper. It must, however, be mentioned, that their fermentation reactions did not differ from the normal types.

On repeated inoculation partly from the smooth, and partly from the rough colonies of the same strain, it was possible to cultivate both the smooth and the rough types in apparently pure form. In the first subcultures smooth colonies were often present amongst the rough, and this probably indicates that not all the elements of the spread colony were rough.

These observations tended to show further that the dense, domed, non-translucent colonies described above were more closely related to the rough colonies than to the smooth.

Chapter IV.

BIOCHEMICAL REACTIONS

1. Nitroso-indole reaction.
2. Fermentation reactions.

1. *The nitroso-indole reaction,* or, as it is more often termed, the cholera-red reaction, is based on the fact that a solution containing both nitrite and indole develops a bright red colour on the addition of a few per cent. of concentrated mineral acid. This reaction may be given by certain bacteria cultured in liquid media, provided they form nitrite and indole during growth.

Most peptone solutions generally contain enough of both nitrate and tryptophane for a bacterial culture producing nitrite and indole to give the cholera-red reaction. A small quantity of nitrate, e. g. potassium nitrate, is, however, often added, to ensure the presence of this substance. Moreover, the amount in peptone of products, the decomposition of which produces indole, may vary; it is therefore advisable, before using the peptone for the examination of the cholera-red reaction of unknown strains, to test it with a culture that is known to form cholera-red.

It should be remembered in using the method, that while it gives information about the presence of nitrite, it does not show whether a reduction of nitrate has on the whole taken place. For bacteria which reduce nitrate beyond the stage of nitrite, will not show this reaction.

When brought forward in 1887, the reaction was said to be specific for the cholera vibrio. It was soon shown, however, that other vibrios also produced the reaction, and it therefore lost some interest, though it was still included in the routine examinations of the characteristics of a vibrio. And, certainly, if positive in every case of a true cholera vibrio, it cannot be said to be entirely without importance.

Author's investigations:

Technique: The examinations were carried out on 18—20 hours' peptone-water cultures, incubated at 37 ° C.. 1 % peptone-water with ½ % sodium chloride was partly used, and partly the same solution with the addition of 0.1 % sodium nitrate. It appeared that a fresh supply of peptone did not contain sufficient nitrate to produce the reaction. To such a culture — containing about 8 cc of medium in an ordinary test tube — a few drops of concentrated hydrochloric acid were added. In the positive cases the red colour appeared within the first few minutes.

The classification of the strains as cholera-red positive, i. e. those that produced the reaction, and cholera-red negative, those that did not produce it, is fairly included in the grouping of the material in Table 16, p. 117, the strains which have a fermentation reaction like type I and II being cholera-red positive, and those which ferment like III, IV, V and VI either positive or negative.

The exceptions to this rule are the strains Nos. 61, 65 and 175, which are cholera-red negative and ferment like type I. Amongst these 3 strains, two — 65 and 175 — are agglutinable with cholera serum. Separate investigations of the nitrite and indole producing power of these 3 strains gave the following results:

	Indole	Nitrite	cholera-red
61	+	0	0
65	+	+	0
175	+	0	0

No. 61 is an old museum-strain, called Vibrio Metchnikow-Bordet.

No. 65, Vibrio Choleræ Pfeifferi, is about 40 years old, and has previously produced cholera-red (Kolle (1894)); it still gives the reaction for each component of the cholera-red reaction. The failure to give the reaction itself is most likely due to quantitative alterations.

No. 175 was isolated on Feb. 2nd 1933. Nothing is known about the cholera-red reaction at that time. The author's investigation was carried out on May 5th 1933.

The remaining cholera-red negative strains were also examined separately as regard their power of producing nitrite and indole. The results were as follows:

	Strain No.
+ nitrite 0 indole	35—78—88—283—293—303—306—318—321—336
0 nitrite + indole	297
0 nitrite 0 indole	24—83

Technique of test for indole: a few drops of the following solution is poured on to a 24 hours' peptone-water culture:

paradimethylamidobenzaldehyd	8 g
96 per cent. alcohol	760 cc
conc. hydrochloric acid	160 cc

The formation of a red colour between the two layers shows the presence of indole.

Test for nitrite: to a 24 hours' culture in peptone-water (or peptone-water with the addition of nitrate), a few drops of each of the following reagents are added: *A:* 0.8 per cent. sulphanilic acid in 5*n* acetic acid. *B:* 0.6 per cent dimethyl-α-naphtylamin in 5*n* acetic acid. The appearance of a red colour after shaking indicates the presence of nitrite.

On the whole it may be said that the nitrose-indole reaction is useful, although not indispensable, in the investigations necessary to identify a vibrio.

2. *Fermentation reactions:*

Although it has been known for long that several vibrios possessed the power of acid formation in different substances, these reactions have so far not been used for the classification of the vibrios, nor are they generally described among the usual characteristics of a vibrio.

This problem was first tackled by Bitter, who showed in 1886 that cholera vibrios were able to acidify saccharose, lævulose, glucose and starch. But only in 1923 was the first attempt made by Nobechi to classify the Vibrio Choleræ and the cholera-like vibrios by their fermentation reactions. Previously Gaehtgens in 1916 had described investigations on the varying time in the appearance of a changing to the original colour of the indicator colour in media fermented by cholera vibrios and cholera-like vibrios.

As the reactions did not appear until the 3rd to 7th day they could not attain practical signifiance, even if they were found to be characteristic. The author did not find these phenomena constant features in the vibrios, as will be seen from the results of the experiments p. 74.

As starch, as mentioned, is fermented by V. Choleræ, it was used in different media specially prepared for the isolation of colonies of cholera vibrios. If this property were specific or nearly so, this mode of cultivation employing a suitable indicator would be very useful — but as numerous other vibrios and other bacterial also can ferment the substance at any rate generally sold under the name of starch, and used by me, the use of this substance in this manner does not seem very practical. An identical view was held by Nobechi in 1923.

Kodama and Takeda in 1923 tried to establish a specific reaction for cholera vibrios, based on the idea that starch fermentation was specific to the cholera vibrio. They used peptone-water with ½ % potato starch as medium . 7—24 hours after the inoculation and incubation at 37° C. a few drops of a watery solution of iodine in potassium iodide were added. If this produced a yellow colour in the medium, the bacterium was supposed to be a cholera vibrio, if

the colour, owing to the presence of starch, became blue, it was not a cholera vibrio. Gildemeister and Herzberg at once proved the non-specificity of the reaction by showing that many other vibrios, several Gram-negative rods, and also a few cocci produced the same reaction as the V. Choleræ.

Nobechi in 1923 attemped by examination of the fermentative powers of 21 cholera vibrios and 8 other vibrios towards 17 different substances to establish a classification. He found that the cholera vibrio and most of the cholera-like vibrios fermented glucose, lævulose, saccharose, galactose, maltose, mannose, dextrine, starch and mannite, producing acid but not gas. Arabinose, xylose, inulin, dulcite, rhamnose and salicin were not fermented, and with lactose and glycerine the effect was variable. One of Nobechi's strains, an old laboratory strain, which was agglutinable by cholera serum, did not, even after repeated subinoculation and animal passage, ferment saccharose. This strain was also non-motile. Nobechi only examined the reactions when peptone-water was used as original medium. As it will be shown later, the original medium may play a rôle in those cases in which a strain cannot form enough acid to turn the indicator.

The results of my fermentation tests agree on the whole with those of Nobechi. Nevertheless I feel justified in drawing up a fermentation scheme, to be used for classification, because my material is far larger, especially in regard to cholera-like strains. This makes certain connections clear, which, in a smaller material, might appear accidental, and from which it would, therefore, not be justifiable to draw conclusions.

In a small investigation in 1929 Stutzer examined the fermentation reactions of 10 vibrios, agglutinable with cholera serum, which had been isolated from fæces, and by using 10 of the media mentioned above, he found results analogous to those of Nobechi.

Kuroya and Oshio reported in December 1933 the results of fermentation tests with 65 strains, mostly isolated at Shanghai. Their results are quite comparable with mine, as 3 of the strains are also included in my material, and the other strains, when examined serologically were grouped against these 3. There is very close accord between the reactions found at Shanghai and my results. The substances investigated were xylose, arabinose, glycogen, galactose,

mannose, saccharose, lactose, maltose, mannite, dextrine, inulin and salicin.

The conclusion of the investigations described above is that the power of acid formation of the V. Choleræ and other vibrios in a series of substances is known, but up to the present this has not justified a grouping of the vibrios.

Author's investigations:

Technique: As original medium 1 per cent. watery solution of Riedel peptone with ½ per cent. sodium chloride was used. To this the substance, in which the power of acid formation was to be examined, was added in a concentration of ½ per cent. As indicator bromthymol-blue was used in a concentration of $^1/_{40}$ per thousand. By adding NaOH the hydrogen ion concentration was fixed between 8 and 8.4. The medium was poured into test tubes 10—12 mm wide, in a layer 4 cm high. For the examinations on the strains Nos. 1—109 an inner tube was used to determine possible gas production. The tubes were inoculated by a drop from a 20—24 hours' bouillon culture.

Investigations: In the first series of experiments the power of acid formation of more than 100 strains was tested in 20 different media, the strains representing vibrios of very different origins.

The substances examined were:

Carbohydrates:	Hexoses: d-glucose, d-lævulose, d-galactose, d-mannose.
	disaccharides: lactose, saccharose, maltose.
	polysaccharides: dextrine, glycogen, starch.
	pentoses: l-arabinose, d-xylose.
	methylpentoses: l-rhamnose.
Alcohols:	triatomic: glycerine.
	pentatomic: adonit.
	hexatomic: d-mannite, i-dulcite, d-sorbite.
Glycosides:	salicin.
Organic acids:	d-tartaric acid.

The tubes were read 4 times during the first 24 hours, namely after 6, 8, 12 and 24 hours respectively, at 37 ° C.. Afterwards they were read once a day for a fortnight, the tubes being kept in the incubator during the whole of this period.

In table 2 are shown the results of the fermentation tests, 0 indicates that the indicator never showed acid reaction, (+) that the colour of the indicator became green or slightly greenish yellow, and + that the indicator turned clear yellow. The turning of bromthymol-blue from blue to yellow takes place at pH 7.5—7. 0 against the tartatic acid tubes, means that the indicator was not decolourized. The figure above and to the right of the marks gives the time in days from the inoculation till the indicator turned yellow — a figure above and to the left of the mark indicates the time when the colour of the indicator *began* to change. Production of gas was not seen in any case. This is not referred to in the table.

Table 2, see p. 58—65.

Table

Fermentation tests,

	Glucose	Lævulose	Galactose	Mannose	Lactose	Saccharose	Maltose	Dextrine	Starch
1.	+	+	+	+	+	+	+	+	+
2.	+	+	+	+	+	+	+	+	+
3.	+	+	+	+	+	+	+	+	+
4.	+	+	+	+	+	+	+	+	+
5.	+	+	+	+	+	+	+	+	+
6.	+	+	+	+	+	+	+	+	+
7.	+	+	+	+	+	+	+	+	+
8.	+	+	+	+	+	+	+	+	+
9.	+	+	+	+	+	+	+	+	+
10.	+	+	+	+	+	+	+	+	+
11.	+	+	+	+	+	+	+	+	+
12.	+	+	+	+	+	+	+	+	+
13.	+	+	+	+	+	+	+	+	+
14.	+	+	+	+	+	+	+	+	+
15.	+	+	+	+	+	+	+	+	+
16.	+	+	+	+	+	+	+	+	+
17.	+	+	+	+	+	+	+	+	+
18.	+	+	+	+	+	+	+	+	+
19.	+	+	+	+	+	+	+	+	+
20.	+	+	+	+	+	+	+	+	+
21.	+	+	+	0	+	+	+	+	+
22.	+	+	+	+	+	+	+	+	+
23.	+	+	+	+	+	+	+	+	+
24.	0	0	0	0	0	0	0	0	0
25.	+	+	+	+	+	+	+	+	+
26.	+	+	+	0	+	+	+	+	+
27.	+	+	+	0	+	+	+	+	+
28.	+	+	+	0	+	+	+	+	+
29.	+	+	+	0	+	+	+	+	+
30.	+	+	+	0	+	+	+	+	+

2.

strains Nos 1—109.

Glycogen	Arabinose	Xylose	Rhamnose	Mannite	Dulcite	Sorbite	Adonite	Glycerine	Salicin	Tartrate
+¼	0	0	0	+⅓	0	0	0	2+7	0	0
+¼	0	0	0	+⅓	0	0	0	2+7	0	0
+¼	0	0	0	+⅓	0	0	0	3+9	0	0
+¼	0	0	0	+½	0	0	0	3+9	0	0
+¼	0	0	0	+⅓	0	0	0	3+6	0	0
+¼	0	0	0	+⅓	0	0	0	3+6	0	0
+1	0	0	0	+⅓	0	0	0	0	0	0
+¼	0	0	0	+⅓	0	0	0	2+9	0	0
+¼	0	0	0	+⅓	0	0	0	2+3	0	0
+⅓	0	0	0	+⅓	0	0	0	3+	0	0
+¼	0	0	0	+1	0	0	0	2+	0	0
+¼	0	0	0	+1	0	0	0	2+3	0	0
+¼	0	0	0	+½	0	0	0	4+	0	0
+¼	0	0	0	+½	0	0	0	2+9	0	0
+¼	0	0	0	+½	0	0	0	4+5	0	0
+¼	0	0	0	+½	0	0	0	2+8	0	0
+¼	0	0	0	+½	0	0	0	3+6	0	0
+¼	0	0	0	+⅓	0	0	0	1+13	0	0
+¼	0	0	0	+½	0	0	0	2+6	0	0
+¼	0	0	0	+½	0	0	0	2+5	0	0
+¼	0	0	0	+¼	0	0	0	2+4	0	0
+¼	0	0	0	+1	0	0	0	3+8	0	0
+¼	0	0	0	+1	0	0	0	2+3	0	0
0	0	0	0	0	0	0	0	0	0	0
+¼	0	0	0	+1	0	0	0	2+5	0	0
+¼	0	0	0	+¼	0	0	0	2+4	0	0
+¼	0	0	0	+⅓	0	0	0	0	0	0
+¼	0	0	0	+¼	0	0	0	0	0	0
+¼	0	0	0	+¼	0	0	0	0	0	0
+¼	0	0	0	+¼	0	0	0	2+	0	0

	Glucose	Lævulose	Galactose	Mannose	Lactose	Saccharose	Maltose	Dextrine	Starch
31.	+ ¼	+ ¼	1 + 2	+ ¼	+ 5	+ ¼	+ ¼	+ ¼	+ ¼
32.	+ ¼	+ ¼	1 + 2	+ ¼	7 + 8	+ ¼	+ ¼	+ ¼	+ ¼
33.	+ ¼	+ ¼	+ 2	+ ¼	0	+ ¼	+ ¼	+ ¼	+ ¼
34.	+ ¼	+ ¼	+ 1	+ ¼	5 + 6	+ ¼	+ ¼	+ ¼	+ ¼
35.	+ ¼	+ ¼	+ ⅓	+ ¼	0	+ ¼	+ ½	+ ¼	+ ¼
36.	+ ¼	+ ¼	½ + 1	+ ¼	9 + 11	+ ¼	+ ¼	+ ¼	+ ¼
37.	+ ¼	+ ¼	+ 1	+ ¼	5 + 6	+ ¼	+ ¼	+ ¼	+ ¼
38.	+ ¼	+ ¼	+ 1	+ ¼	5 + 6	+ ¼	+ ¼	+ ¼	+ ¼
39.	+ ¼	+ ¼	+ 1	0	+ 4	+ ¼	+ ¼	+ ¼	+ ¼
40.	+ ¼	+ ¼	1 + 2	+ ½	9 + 10	0	+ ¼	+ ¼	½ + 3
41.	+ ¼	+ ¼	1 + 2	+ ¼	5 + 7	+ ¼	+ ¼	+ ¼	+ ¼
42.	+ ¼	+ ¼	1 + 2	+ ¼	5 + 6	+ ¼	+ ¼	+ ¼	+ ¼
43.	+ ¼	+ ¼	+ 3	+ ¼	4 + 5	+ ¼	+ ¼	+ ¼	+ ¼
44.	+ ¼	+ ¼	1 +	+ ¼	5 + 9	+ ¼	+ ¼	+ ¼	+ ¼
45.	+ ¼	+ ¼	2 + 3	+ ¼	4 + 5	+ ¼	+ ¼	+ ¼	+ ¼
46.	+ ¼	+ ¼	+ 1	+ ¼	5 + 6	+ ¼	+ ¼	+ ¼	+ ¼
47.	+ ¼	+ ¼	1 + 2	+ ¼	6 + 7	+ ¼	+ ¼	+ ¼	+ ¼
48.	+ ¼	+ ¼	2 + 3	+ ¼	6 + 7	+ ¼	+ ¼	+ ¼	+ ¼
49.	+ ¼	+ ¼	3 + 4	+ ¼	4 + 6	+ ¼	+ ¼	+ ¼	+ ¼
50.	+ ¼	+ ¼	3 + 4	+ ¼	0	+ ¼	+ ¼	+ ¼	+ ¼
51.	+ ¼	+ ¼	+ 2	+ ¼	5 + 7	+ ¼	+ ¼	+ ¼	+ ¼
52.	+ ¼	+ ¼	1 + 2	+ ¼	5 + 6	+ ¼	+ ¼	+ ¼	+ ¼
53.	+ ¼	+ ¼	2 + 4	+ ¼	0	+ ¼	+ ¼	+ ¼	+ ¼
54.	+ ¼	+ ½	0	+ ½	0	+ ½	+ 1	+ ½	+ ½
55.	+ ¼	+ ¼	1 + 5	+ ¼	4 + 8	+ ¼	+ ¼	+ ¼	+ ¼
56.	+ ¼	+ ¼	1 + 3	+ ¼	5 + 6	+ ¼	+ ⅓	+ ¼	+ ¼
57.	+ ¼	+ ¼	2 + 4	+ ¼	+ 5	+ ¼	+ ¼	+ ¼	+ ¼
58.	+ ¼	+ ¼	2 + 4	+ ¼	+ 5	+ ¼	+ ¼	+ ¼	+ ¼
59.	+ ½	+ 2	0	+ 2	0	+ 2	0	0	0
60.	+ ¼	+ ¼	4 +	0	0	+ ¼	+ ¼	+ ¼	+ ¼
61.	+ ⅓	½ + 1	2 + 4	+ ½	+ 12	+ ½	+ 1	+ ½	+ ¼
62.	+ ¼	+ ⅓	3 + 4	+ ⅓	6 + 7	+ ⅓	+ ⅓	+ ⅓	+ ¼
64.	+ ¼	+ ¼	5 + 6	+ ¼	6 + 7	+ ¼	+ ¼	+ ¼	+ ¼

Glycogen	Arabinose	Xylose	Rhamnose	Mannite	Dulcite	Sorbite	Adonite	Glycerine	Salicin	Tartrate
+¼	0	0	0	+½	0	0	0	2+3	0	0
+¼	0	0	0	+½	0	0	0	2+5	0	0
+¼	0	0	0	+¼	0	0	0	2+3	0	0
+¼	0	0	0	+½	0	0	0	2+4	0	0
+¼	+⅓	0	0	+¼	0	0	0	2+4	0	0
+¼	0	0	0	+1	0	0	0	2+3	0	0
+¼	0	0	0	+½	0	0	0	2+9	0	0
+¼	0	0	0	+½	0	0	0	2+9	0	0
+¼	0	0	0	+¼	0	0	0	2+	0	0
½+	0	0	0	+¼	0	0	0	2+10	0	0
+¼	0	0	0	+½	0	0	0	2+9	0	0
+¼	0	0	0	+½	0	0	0	2+11	0	0
+¼	0	0	0	+¼	0	0	0	5+6	0	0
+¼	0	0	0	+¼	0	0	0	2+3	0	0
+¼	0	0	0	+¼	0	0	0	2+	0	0
+¼	0	0	0	+½	0	0	0	2+12	0	0
+¼	0	0	0	+1	0	0	0	3+10	0	0
+¼	0	0	0	+½	0	0	0	2+3	0	0
+¼	0	0	0	+½	0	0	0	2+6	0	0
+¼	0	0	0	+½	0	0	0	3+7	0	0
+¼	0	0	0	+½	0	0	0	3+6	0	0
+¼	0	0	0	+½	0	0	0	2+6	0	0
+¼	0	0	0	+1	0	0	0	2+3	0	0
+½	0	0	0	+2	0	0	0	0	0	0
+¼	0	0	0	+1	0	0	0	7+8	0	0
+¼	0	0	0	+½	0	0	0	2+3	0	0
+¼	0	0	0	+¼	0	0	0	2+4	0	0
+¼	0	0	0	+½	0	0	0	2+4	0	0
0	0	0	0	0	0	0	0	0	0	0
+¼	0	0	0	+⅓	0	0	0	3+8	0	0
½+1	0	0	0	+1	0	0	0	+½	+12	0
+⅓	0	0	0	+2	0	0	0	3+	0	0
+¼	0	0	0	+1	0	0	0	0	0	0

	Glucose	Lævulose	Galactose	Mannose	Lactose	Saccharose	Maltose	Dextrine	Starch
65.	+ ½	+ ⅓	3 + 4	+ ½	0	+ ½	+ ½	+ ½	½ + 1
66.	+ ¼	+ ⅓	0	+ 1	0	+ ¼	+ ⅓	+ ½	1 +
67.	+ ¼	+ ¼	1 + 2	+ ¼	6 + 7	+ ¼	+ ⅓	+ ¼	+ ¼
68.	+ ¼	+ ¼	½ + 1	0(+ ⅓)*)	+ 5	+ ¼	+ ¼	+ ¼	+ ½
69.	+ ⅓	+ ½	1 + 2	+ ½	0	+ ⅓	+ ½	+ ½	+ ½
70.	+ ¼	+ 1	7 +	+ 1	0	+ ⅓	+ 1	+ ½	+ ½
71.	+ ¼	+ ¼	+ 3	+ ¼	6 + 11	+ ¼	+ ⅓	+ ¼	+ ¼
72.	+ ¼	+ ¼	+ 1	0	8 + 11	+ ¼	+ ⅓	+ ¼	+ ¼
73.	+ ¼	+ ¼	+ ½	0	0	+ ¼	+ ¼	+ ¼	+ ¼
74.	+ ¼	+ ⅓	0	+ ⅓	10 + 12	+ ⅓	+ ¼	+ ⅓	+ ⅓
75.	+ ¼	+ ⅓	1 + 6	0	0	+ ⅓	+ ¼	+ ⅓	+ ⅓
76.	+ ¼	+ ½	+ 2	+ ½	0	+ ⅓	+ ⅓	+ ½	+ ½
78.	0	0	0	0	0	0	0	0	0
79.	+ ¼	+ ¼	1 + 2	0	0	+ ¼	+ ¼	+ ¼	+ ⅓
80.	+ ⅓	+ ½	+ 1	+ ½	0	+ ½	+ ½	+ ½	+ ½
81.	+ ¼	+ ¼	+ 2	+ ½	4 + 5	+ ¼	+ ¼	+ ¼	+ ¼
82.	+ ⅓	+ ½	½ + 1	+ ½	0	+ ½	½ + 1	½ + 1	1 +
83.	0	0	0	0	0	0	0	0	0
84.	+ ½	+ ½	+ 3	0	12 + 13	+ ½	½ + 1	+ ½	+ ½
85.	+ ¼	+ ¼	½ + 1	0	0	+ ¼	+ ¼	+ ¼	+ ¼
86.	+ ⅓	+ ⅓	1 + 3	0	+ 8	+ ⅓	+ ⅓	+ ½	+ ½
87.	+ ¼	+ ¼	1 + 2	+ ¼	6 + 7	+ ¼	+ ¼	+ ¼	+ ¼
88.	+ ¼	+ ¼	+ ½	+ ¼	0	+ ¼	+ ¼	+ ¼	+ ¼
89.**	+ 3	+ 3	+ 3	+ 3	0	+ ¼	+ ¼	+ ¼	+ ¼
90.	+ ¼	+ ¼	+ 2	+ ¼	6 + 7	+ 3	+ 3	+ 3	+ 3
91.	+ ¼	+ ¼	+ 1	+ ¼	6 + 7	+ ¼	+ ¼	+ ¼	+ ¼
93.	+ ¼	+ ¼	+ 2	0	0	+ ¼	+ ¼	+ ¼	+ ¼
94.	+ ¼	+ ¼	+ 1	+ ¼	12 + 13	+ ¼	+ ¼	+ ¼	+ ¼

*) On repeated examination strain 68 did not ferment mannose. It seems most likely that the results put down for the first experiment was due to an experimental error.

**) The strain, which has lived as laboratory strain for about 30 years, grows very slowly and torpidly.

Glycogen	Arabinose	Xylose	Rhamnose	Mannite	Dulcite	Sorbite	Adonite	Glycerine	Salicin	Tartrate
½+[1]	0	0	0	+½	0	0	0	0	0	0
[1]+[3]	0	0	0	−[2]	0	0	0	0	0	0
+¼	0	0	0	+[1]	0	0	0	[3]+	0	0
+¼	0	0	0	+⅓	0	0	0	[3]+[7]	0	0
+¼	0	0	0	+½	0	0	0	[3]+	0	0
+½	0	0	0	+½	0	0	0	+[6]	0	0
+¼	0	0	0	+⅓	0	0	0	[2]+	0	0
+¼	+⅓	0	0	+⅓	0	0	0	[2]+	0	0
+¼	0	0	0	+¼	0	0	0	[2]+	0	0
+⅓	0	0	0	+[1]	0	0	0	+[2]	0	0
+⅓	0	0	0	+½	0	0	0	[3]+[7]	0	0
+½	0	0	0	[2]+[4]	0	0	0	[3]+[6]	0	0
0	0	0	0	0	0	0	0	0	0	0
+¼	0	0	0	+¼	0	0	0	[2]+[3]	0	0
+½	0	0	0	0	0	0	0	0	0	0
+¼	0	0	0	+¼	0	0	0	[4]+[5]	0	0
+½	0	0	0	+[4]	0	0	0	[4]+[6]	0	0
0	0	0	0	0	0	0	0	0	0	0
+½	0	0	0	+[1]	0	0	0	0	0	0
+¼	0	0	0	+¼	0	0	0	[8]+	0	0
+⅓	0	0	0	+½	0	0	0	0	0	0
+¼	0	0	0	+⅓	0	0	0	[3]+[5]	0	0
+¼	+¼	0	0	+¼	0	0	0	0	0	0
+[3]	0	0	0	0	0	0	0	0	0	0
+¼	0	0	0	+½	0	0	0	+[3]	0	0
+¼	0	0	0	+½	0	0	0	[3]+[6]	0	0
+¼	0	0	0	+¼	0	0	0	[2]+	0	0
+¼	0	0	0	+½	0	0	0	[2]+[5]	0	0

	Glucose	Lævulose	Galactose	Mannose	Lactose	Saccharose	Maltose	Dextrine	Starch
95.	+ ¼	+ ¼	2 + 3	+ ¼	5 + 6	+ ¼	+ ¼	+ ¼	+ ¼
96.	+ ¼	+ ¼	2 + 3	+ ¼	6 + 7	+ ¼	+ ⅓	+ ¼	+ ¼
97.	+ ¼	+ ¼	2 + 3	+ ¼	6 + 9	+ ¼	+ ¼	+ ¼	+ ¼
98.	+ ¼	+ ¼	+ 2	+ ¼	+ 5	+ ¼	½ +	+ ¼	+ ¼
99.	+ ½	+ ¼	1 + 3	+ ¼	6 + 7	+ ¼	½ + 1	+ ¼	+ ¼
100.	+ ¼	+ ¼	2 + 3	+ ¼	4 + 5	+ ¼	+ ½	+ ¼	+ ¼
101.	+ ¼	+ ¼	2 + 5	+ ¼	4 + 6	+ ¼	+ ½	+ ⅓	+ ¼
102.	+ ¼	+ ¼	+ 2	+ ⅓	3 + 4	+ ¼	+ ¼	+ ⅓	+ ¼
103.	+ ¼	+ ¼	+ 2	+ ¼	5 + 6	+ ¼	+ ¼	+ ¼	+ ¼
104.	+ ¼	+ ¼	+ 2	0	5 + 6	+ ¼	+ ¼	+ ¼	+ ¼
105.	+ ¼	+ ¼	1 + 2	+ ⅓	4 + 5	+ ¼	+ ¼	+ ¼	+ ¼
106.	+ ¼	+ ¼	+ 3	+ ¼	6 +	(0) +	+ ⅓	+ ¼	+ ¼
107.	+ ¼	+ ¼	1 + 2	+ ¼	5 + 7	+ ¼	+ ¼	+ ¼	+ ¼
108.	+ ¼	+ ¼	1 + 2	+ ¼	5 + 6	+ ¼	+ ¼	+ ⅓	+ ¼
109.	+ ¼	+ ¼	+ ⅓	0	7 +	+ ¼	+ ¼	+ ¼	+ ¼

Glycogen	Arabinose	Xylose	Rhamnose	Mannite	Dulcite	Sorbite	Adonite	Glycerine	Salicin	Tartrate
+¼	0	0	0	+½	0	0	0	0	0	0
+¼	0	0	0	½+1	0	0	0	5 ⊢	0	0
⊦¼	0	0	0	½ ⊣ ·1	0	0	0	6 ⊦7	0	0
⊣¼	0	0	0	−½	0	0	0	2+5	0	0
+¼	0	0	0	+½	0	0	0	6+	0	0
+¼	0	0	0	⊦½	0	0	0	0	0	0
+¼	0	0	0	⊦ ⅓	0	0	0	2⊣	0	0
+¼	0	0	0	⊔¼	0	0	0	2+6	0	0
⊦¼	0	0	0	+⅓	0	0	0	2+7	0	0
⊣¼	0	0	0	⊢¼	0	0	0	2+6	0	0
+¼	0	0	0	⊣¼	0	0	0	3+5	0	0
+¼	0	0	0	+⅓	0	0	0	2+	0	0
⊢¼	0	0	0	⊢⅓	0	0	0	3⊣	0	0
⊣¼	0	0	0	⊣½	0	0	0	4+	0	0
+¼	0	0	0	⊣¼	0	0	0	2 ⊦5	0	0

From the table it will be seen that several substances — glucose, lævulose, maltose, dextrine, glycogen, starch, mannite — are decomposed by nearly all the vibrios with acid formation in ¼—½ day. Galactose is generally decomposed in 2—3 days, whilst lactose and glycerine are not fermented till the 4th—12th day or not at all. Xylose, rhamnose, adonite, dulcite, sorbite, tartaric acid and, with a single exception, salicin, are not fermented.

Only towards 3 substances do the reactions show a behaviour which at first sight seems to form a reasonable criterion for a classification of the vibrios examined. These 3 substances are: mannose (Mn), saccharose (Sa) and arabinose (Ar). If fermentation appears in these substances it takes place within 6—12 hours. Only 4 strains took longer: strains 66 and 70 (12—24 hours), strain 59, V. Nasik, which on the whole has distinctive fermentation reactions (2 days), and strain 89, which is a laboratory strain about 30 years old (3 days). One strain, which at first did not decompose saccharose, did so after a few subinoculations.

Apart from the reaction with these three substances, the fermentation results with galactose, lactose and glycerine might also form a basis for classification. With galactose, fermentation most often took place on the 2nd—3rd day; in a few cases before this — as early as 8 hours, in some later — on the 6th—7th day. Moreover a few strains did not ferment galactose. These might, of course, be placed in a separate group, but so far I have not been able to find here a basis for simple grouping of vibrios — and a reaction that does not appear until the 6th or 7th day will hardly assume practical significance.

In regard to lactose there seemed no rule for the "turning time" of the indicator; it occurred any time between 3—14 days, and in several strains the indicator did not turn at all within the period of the experiment. In these circumstances this carbohydrate must be excluded as a possible basis for classification. Glycerine shows entirely similar relations.

If some strains had fermented comparatively quickly e. g. in 1—3 days, and the rest comparatively late, e. g. in 10—15 days, a grouping into quickly-fermenting and late-fermenting strains might possibly have been of practical significance. A single strain, No. 61, in repeated experiments, ferments salicin in 11—13 days, a property

characteristic of this strain. Serologically it appears to hold an exceptional position in the present series. If the vibrios are classified according to their fermentation of mannose, saccharose and arabinose, the strains examined show the distribution indicated in Table 3.

Table 3.

type of fermentation	fermentation	Strain No.	total number of strains.
I	+ *Mn* + *Sa* 0 *Ar*	1—2—3—4—5—6—7—8—9—10—11—12—13—14—15—16—17—18—19—20—22—23—25—31—32—33—34—36—37—38—41—42—43—44—45—46—47—48—49—50—51—52—53—54—55—56—57—58(59)61—62—64—65—66—67—69—70—71—74—76—80—81—82—87—89—90—91—94—95—96—97—98—99—100—101—102—103—105—106—107—108	81
II	0 *Mn* + *Sa* 0 *Ar*	21—26—27—28—29—30—39—60—68—73—75—79—84—85—86—93—104—109	18
III	+ *Mn* + *Sa* + *Ar*	35—88	2
IV	0 *Mn* + *Sa* + *Ar*	72	1
V	+ *Mn* 0 *Sa* 0 *Ar*	40	1
VI	0 *Mn* 0 *Sa* 0 *Ar*	24—78—83	3
Discarded		63—77—92	3

Strains 1—109 distributed according to fermentation type.

The remaining strains of the series, up to No. 408, have been examined only for their ability to form acid in the 3 carbohydrates mentioned; moreover the reactions were not read later than the third day.

Table 4 gives the results of this investigation. In this table a simple + indicates that the turning of the indicator took place within the first 8 hours at 37° C.. A column has been added, giving the grouping (type) in which the fermentation resulted.

Table 4.

Fermentation tests N^os 110—408.

Strain	Mannose	Saccharose	Arabinose	Type of fermentation	Strain	Mannose	Saccharose	Arabinose	Type of fermentation	Strain	Mannose	Saccharose	Arabinose	Type of fermentation
110	+	+	0	I	130	+	+	0	I	151	+	+	0	I
112	0	+	0	II	131	+	+	0	I	152	+	+	0	I
113	+	+	0	I	132	+	+	0	I	153	+	+	0	I
114	+	+	0	I	133	+	+	0	I	154	+	+	0	I
115	+	+	0	I	134	+	+	0	I	155	+	+	0	I
116	+	+	0	I	135	+	+	0	I	156	+	+	0	I
117	+	+	0	I	136	+	+	0	I	157	+	+	0	I
118	+	+	0	I	137	+	+	0	I	158	+	+	0	I
119	+	+	0	I	138	+	+	0	I	159	0	+	0	II
120	+	+	0	I	139	+	+	0	I	160	+	+	0	I
121	+	+	0	I	140	+	+	0	I	161	+	+	0	I
122	+	+	0	I	142	+	+	0	I	162	+	+	0	I
123	+	+	0	I	143	+	+	0	I	163	+	+	0	I
124	+	+	0	I	144	+	+	0	I	164	+	+	0	I
125	+	+	0	I	146	+	+	0	I	165	+	+	0	I
126	+	+	0	I	147	+	+	0	I	166	+	+	0	I
127	+	+	0	I	148	+	+	0	I	167	+	+	0	I
128	+	+	0	I	149	+	+	0	I	168	+	+	0	I
129	+	+	0	I	150	+	+	0	I	169	+	+	0	I

Strain	Mannose	Saccharose	Arabinose	Type of fermentation	Strain	Mannose	Saccharose	Arabinose	Type of fermentation	Strain	Mannose	Saccharose	Arabinose	Type of fermentation
170	+	+	0	I	203	+	+	0	I	237	+	+	0	I
171	+	+	0	I	204	0	+	0	II	239	+	+	0	I
172	+	+	0	I	206	+	+	0	I	240	+	+	0	I
173	+	+	0	I	207	+	+	0	I	241	+	+	0	I
174	+	+	0	I	208	+	+	0	I	242	+	+	0	I
175	+	+	0	I	209	+	+	0	I	243	+	+	0	I
176	+	+	0	I	210	+	+	0	I	244	+	+	0	I
177	+	+	0	I	211	+	+	0	I	245	+	+	0	I
178	+	+	0	I	212	+	+	0	I	246	+	+	0	I
179	+	+	0	I	213	+	+	0	I	247	+	+	0	I
180	+	+	0	I	214	+	+	0	I	248	+	+	0	I
181	+	+	0	I	215	+	+	0	I	249	+	+	0	I
183	+	+	0	I	216	+	+	0	I	250	+	+	0	I
184	+	+	0	I	217	+	+	0	I	251	0	0	0	VI
185	+	+	0	I	218	+	+	0	I	252	+	+	0	I
186	+	+	0	I	219	+	+	0	I	253	+	+	0	I
187	+	+	0	I	220	+[2]	+[2]	0	I	254	0	+	0	II
188	+	+	0	I	221	+	+	0	I	256	+	+	0	I
189	+	+	0	I	223	+	+	0	I	257	+	+	0	I
190	+	+	0	I	224	+	+	0	I	258	+	+	0	I
191	+	+	0	I	225	+	+	0	I	259	0	+	0	II
192	+	+	0	I	226	+	+	0	I	260	+	+	0	I
193	+	+	0	I	227	+	+	0	I	261	+	+	0	I
194	+	+	0	I	228	+	+	0	I	262	+	+	0	I
195	+	+	0	I	229	+	+	0	I	263	+	+	0	I
196	+	+	0	I	230	+	+	0	I	264	+	+	0	I
197	+	+	0	I	231	+	+	0	I	266	+	+	0	I
198	+	+	0	I	232	+	+	0	I	267	0	+	0	II
199	+	+	0	I	233	+	+	0	I	268	+	+	0	I
200	+	+	0	I	234	+	+	0	I	269	+	+	0	I
201	+	+	0	I	235	+	+	0	I	271	+	+	0	I
202	+	+	0	I	236	+	+	0	I	275	+	+	0	I

Strain	Mannose	Saccharose	Arabinose	Type of fermentation
276	+	+	0	I
277	+	+	0	I
279	+	+	0	I
280	+	+	0	I
281	+	+	0	I
282	+	+	0	I
283	+	+	+	III
284	0	+	0	II
285	0	+	0	II
286	+	+	0	I
287	+	+	0	I
288	0	+	0	II
289	+	+	0	I
290	+	+	0	I
291	+	+	0	I
292	+	+	0	I
293	+	+	+[1]	III
294	+	+	0	I
295	+	+	+	III
296	0	+	0	II
297	0	0	0	VI
298	0	+	0	II
299	0	+	0	II
300	0	+	0	II
301	+	+	0	I
302	0	+	0	II
303	+	+	+	III
304	0	+	0	II
305	0	+	+	IV
306	+	+	+	III
307	0	+	0	II
308	0	+	0	II
309	+	+	0	I
310	0	+	0	II
311	+	+	0	I
312	0	+	0	II
313	+	+	0	I
314	+	+	0	I
316	+	+	0	I
317	+	+	0	I
318	+	+	+	III
320	0	+	0	II
321	+	+	+	III
322	+	+	0	I
323	0	+	0	II
324	0	+	0	II
325	+	+	0	I
327	0	+	0	II
328	0	+	0	II
329	0	+	0	II
330	0	+	0	II
331	0	+	0	II
332	0	+	0	II
333	+	+	0	I
334	+	+	0	I
335	0	+	0	II
336	+	+	+[1]	III
337	0	+	0	II
338	0	+	0	II
339	0	+	0	II
340	+	+	0	I
341	0	+	0	II
342	+	+	+	III
344	0	+	0	II
345	0	+	0	II
346	0	+	0	II
347	0	+	0	II
348	0	+	0	II
349	0	+	+[1]	IV
350	0	+	0	II
351	+[1]	+	+[1]	III
352	0	+	0	II
353	0	+	0	II
354	+	0	0	V
355	0	+	0	II
356	0	+	0	II
357	0	+	0	II
359	+	+	0	I
360	+	+	0	I
361	+	+	0	I
363	+	+	0	I
364	+	+	0	I
365	+	+	0	I
366	+	+	0	I
368	+	+	0	I
369	+	+	0	I
370	+	+	0	I
371	+	+	0	I
372	+	+	0	I
373	+	+	0	I
374	+	+	0	I
375	+	+	0	I
376	+	+	0	I
377	+	+	0	I
378	+	+	0	I
379	+	+	0	I

Strain	Mannose	Saccharose	Arabinose	Type of fermentation	Strain	Mannose	Saccharose	Arabinose	Type of fermentation	Strain	Mannose	Saccharose	Arabinose	Type of fermentation
380	0	+	0	II	389	0	+	0	II	400	+	+	0	I
381	0	+	0	II	390	+	+	0	I	401	+	+	0	I
382	0	+	0	II	391	+	+	0	I	402	+	+	0	I
383	0	+	0	II	392	+	+	0	I	403	+	+	0	I
384	+	+	0	I	393	0	+	0	II	404	+	+	0	I
385	+	+	0	I	394	0	+	0	II	405	+	+	0	I
386	0	+	0	II	397	+	+	0	I	407	0	+	0	II
387	0	+	0	II	398	+	+	0	I	408	0	+	0	II
388	0	+	0	II	399	+	+	0	I					

The number of strains belonging to each grouping (type) of fermentation reactions are given in Table 5.

Table 5, see p. 72.

The fermentation reactions of the strains 110 to 408 confirmed the hypothesis put forward on the basis of the preliminary fermentation experiments — that these 3 carbohydrates might be used for a classification of the vibrios, which can all be placed within these groups. Theoretically the 3 carbohydrates allow the formation of two further types of fermentation reactions — + Mn, 0 Sa, + Ar and 0 Mn, 0 Sa, + Ar — but none of the strains examined showed such types of fermentation.

The types IV, V and VI are comparatively small numerically; if in practice the number of carbohydrates used were reduced to 2 or even one, this would have to be done at the cost of saccharose or arabinose, as it is mannose which distinguishes between the two largest groups in the classification. If arabinose were excluded, Type III would become identical with Type I and Type IV with Type II, whilst the difference between the new types I and II and the old V and VI would still exist. Hereby the largest group but two in the classification would disappear and be included in Type I; but, as

Table 5.

		Number of strains
Type I	+ Mannose + Saccharose 0 Arabinose	287
Type II	0 Mannose + Saccharose 0 Arabinose	75
Type III	+ Mannose + Saccharose + Arabinose	12
Type IV	0 Mannose + Saccharose + Arabinose	3
Type V	+ Mannose 0 Saccharose 0 Arabinose	2
Type VI	0 Mannose 0 Saccharose 0 Arabinose	5
	Discarded:	24

Distribution between the different types of fermentation of the 384 strains examined.

will be seen from the serological experiments, it is very important that this Type is not "infected" with strange elements.

If saccharose were excluded, Type V would become identical with Type I, and Type VI identical with Type II. With regard to the first manipulation the same considerations must prevail as in the case of arabinose concerning the "infection" of Type I with strange elements — the second manipulation on the other hand, is of no great importance.

Finally, a comparison of the biochemical and serological reactions shows, that if for practical reasons the classification is to be

simplified, it would be most reasonable to go as far as to exclude both Sa and Ar. It is then possible to place the vibrios in two groups only: the mannose-positive and the mannose-negative vibrios. While the disadvantages resulting from exclusion of each of these two carbohydrates are totalled, the simplification is considerable. Further investigations on the pathogenecity and epidemiological features of the vibrios may possibly show that for practical diagnosis it is sufficient to use one carbohydrate and only two types of fermentation.

But at present I believe that in investigating vibrios it is not justified to reject the help to be obtained from examination for the six groupings of fermentation reactions.

In order to examine the constancy of the property to form acid, 100 strains belonging to different types of fermentation reactions were re-examined 6—14 months after the first investigation with mannose, saccharose and arabinose. Except in a single case — strain 68 — every one of these 100 strains gave findings identical with those of the first investigation. According to the notes, in the first investigation strain No. 68 was able to ferment mannose in 6 hours — but on re-examination 14 months later, it did not ferment this carbohydrate at all. As the strain had been received from the National Collection of Type Cultures, a new "sample" was asked for. This new sample also turned out to be mannose-negative. Because of this and in view of the constancy found in the rest of the strains, I consider the first finding most probably due to an experimental error — the tube read as mannose either contained another carbohydrate, or was contaminated.

It has previously been mentioned that strain 106 could not ferment saccharose until it had been subinoculated several times. This departure from the general rule even in regard to saccharose perhaps indicates that the saccharose-negative strain found by Nobechi was one that had lost its fermentative powers.

As strain 61 — V. Metchnikow-Bordet — fermented salicin in 12—14 days and otherwise fermented like type I, all the strains in Type I that did not agglutinate with cholera serum were examined as to their fermentation towards this substance. It was found that within 14 days none of the strains could form acid in salicin.

As mentioned above, Gaehtgens thought that it was possible to distinguish between cholera vibrios and cholera-like vibrios by

noting the time taken for the indicator to turn back from acid to neutral or alkaline reaction. Gaehtgens used for his experiments agar slopes with the addition of sugar. The fact that I used the fermentation in peptone-water for comparative investigations should really make little difference.

From daily readings I received the impression that the turning back of the indicator was not a constant property of the vibrios, but that it depended more probably on some external factor. In order to investigate this question, 3 strains were chosen which in their behaviour to starch had shown the fermentation reactions indicated in table 6.

Table 6.

day of experiment		1/2	1	2	3	4	5	6	7	14
strain N°	13	+	(+)	0	0	0	0	0	0	0
—	— 18	+	+	+	+	+	+	+	+	+
—	— 99	+	+	0	0	0	0	0	0	0

0 indicates a blue colour of the indicator (pH greater than 7.5).
(+) indicates a greenish yellow colour of the indicator (pH about 6—7).
+ indicates a yellow colour of the indicator (pH less than 6).

All three strains are agglutinable with cholera serum. It will be seen that the indicator does not turn back with strain 18, with strain 99 it turns back in two days and with strain 13 the turning back begins within the first 24 hours.

The tests were now carried out in 4 different series:

1. series: exactly like the first investigation.
2. series: the cotton wool stopper was put in very loosely.
3. series: the stopper was sealed with paraffin.
4. series: ordinary conditions, but 3 4 times the ordinary quantity of culture was used for inoculation.

In all the tubes the colour of the indicator turned from blue to yellow within 8 hours, and remained yellow for the next two weeks. The first series, when compared with the previous investigation,

shows that even under conditions as far as possible identical the phenomenon does not appear constantly in the same strain. As this confirmed the general impression I had gained as to the time required for the indicator to turn back, I did not carry out any further work on the question.

In a smaller series of vibrios the effects in glycerinefuchsin-bouillon according to Stern and in bouillon with the addition of urea were investigated. The indicator did not turn in the first 24 hours.

It has already been mentioned, that in lactose the turning time of the indicator varied greatly. As some of the strains did not ferment lactose within the period of the experiment, an investigation was carried out to determine whether this negative property was of an absolute or relative character. Preliminary tests carried out before the systematic fermentation experiments in order to find a suitable medium, had shown that the turning time of the indicator with the strains that fermented lactose to some degree depended on the principal constituent of the medium. As Riedel peptone was in this respect found to be only fairly satisfactory as compared with extract bouillon and Parke Davis peptone in which the turning of the indicator took place more quickly, the lactose-negative strains — with the exception of the strains belonging to type VI — were examined as to their fermentation capacity towards lactose, using the latter two substances as the chief constituent. By means of this it was found that only the strains 59, 60 and 62 were lactose-negative, whilst the strains previously lactose-negative, formed acid in these media.

Although the behaviour to mannose, in which the indicator turned either in the first 24 hours or not at all, cannot be compared with the late turning of the indicator in lactose, because of the great importance attached to mannose, I felt that here also it was necessary to examine the possible fermentation reactions under optimal conditions. Therefore, the mannose negative strains of the first 109 strains were re-examined with mannose in extract bouillon and Parke Davis peptone. This investigation confirmed the observations with Riedel peptone: none of these strains could decompose mannose with acid formation.

It has already been stated that the rod-shaped strains of the ma-

terial were included in the investigation. As the examination of the fermentation reactions of the first 100 strains or so towards 20 different substances had shown fairly similar types in all the strains, except as regard their behaviour to mannose, saccharose and arabinose, all the rod-shaped strains were investigated with these 20 substances, in order to examine the possibility of excluding some of these strains, either because they showed fermentation reactions different from the typical reactions of the vibrios, or because they could be identified with other biochemically known bacterial types. The chief constituent of these media was extract bouillon with ½ per cent. Liebig's meat extract, 1 per cent. Riedel peptone and ½ per cent. sodium chloride. The carbohydrate was added in a concentration of ½ per cent.. The tests were read after 12, 24, 48 and 72 hours at 37° C., then several times in the interval until 14 days after being put up, when they were read for the last time.

The 23 strains examined were:

24—67—75—78—134—167—174—183—187—188—206—268—284—295—322—342—349—350—354—390—392—394—398.

In excluding the three type-determining carbohydrates, exceptions from the ordinary fermentation types of the vibrios were seen only in the strains 24, 78 and 342. The first two were previously described as not fermenting carbohydrate at all. The third, strain 342, which has been grouped as type III, fermented with gas production; as it also ferments lactose and gives the reaction for indole, it appears to be B. coli.

Thus, only 3 of the 23 rod-shaped strains differ in their biochemical reactions from the ordinary fermentation type of the vibrios. Of these 3, only one strain may be definitely excluded, for the strains 24 and 78 might be variation forms of the vibrio described as V. alkaligenes (Baerthlein).

Conclusion: With the fermentation reactions carried out in this investigation as a basis, it may be stated that numerous vibrios show the same behaviour towards several different substances. Of 20 different substances there were only three (mannose, saccharose and arabinose) towards which the vibrios showed such variations in behaviour that these reactions could be used as a criterion for a

classification of the vibrios based on the fermentation tests. On the basis of acid-formation in these three substances, 6 different types of fermentation grouping were established:

	Mannose	Saccharose	Arabinose
Type I	+	+	0
— II	0	+	0
— III	+	+	+
— IV	0	+	+
— V	+	0	0
— VI	0	0	0

where + indicates formation of acid and 0 no formation of acid.

384 vibrios, more or less collected at random, were grouped as follows:

type	I	II	III	IV	V	VI
number of strains	287	75	12	3	2	5

None of the vibrios examined in this connection have shown production of gas with the acid formation.

Chapter V.

INVESTIGATIONS ON HÆMOLYSIS

As mentioned in the introduction, the importance of hæmolysis for the identification of a vibrio has been constantly disputed ever since the phenomenon was first observed, and has caused the division of bacteriologists into two camps. At first purely technical factors were the main cause of disagreement. In 1906, when the problem first arose, and during the following years investigators generally used blood agar for the observation of hæmolysis, but, as will be shown below, this medium is not suitable for this purpose. Another factor which may cause varying results is the animal from which the blood is taken. Vibrios which cause strong hæmolysis with rabbit blood corpuscles, may not be able to hæmolyse goat blood corpuscles at all.

Historically the problem of hæmolysis is connected with the strains isolated at El Tor. Investigating a larger material of vibrios to day, one soon discovers that strains agglutinable with cholera serum, other than those isolated at El Tor, may also cause strong hæmolysis. The reason why the El Tor strains are of special importance is because, strains agglutinable with cholera serum have several times been isolated at El Tor, during cholera-free periods, and contrary to what is generally found in vibrios agglutinable with this serum, they were hæmolytic.

In 1905 F. Gotschlich at the quarantine station El Tor found vibrios in the bodies of 38 out of 107 pilgrims who had died with symptoms of dysentery. Of these 38 strains, 6 were agglutinable with cholera serum, and neither morphologically nor by cultivation were they distinguishable from cholera vibrios. He believed that they were true cholera vibrios, and explained the fact that no epidemic had ensued by assuming that a factor causing profuse diarrhoea

in the first case had been missing, and this would prevent the dissemination of the infectious substance. The cholera vibrio is only slightly resistant, and in each of the cholera carriers only few vibrios were found. In his arguments Gotschlich overlooks the fact that the vibrios were also found in several cases of dysentery.

In the papers of Kraus and Pribram dealing with these problems, it was also shown that the El Tor strains formed a hæmolysin which, by immunization experiments, was found to be identical with a hæmolysin formed by a hæmolytic strain which was *not* agglutinable with cholera serum. Almost at the same time, Mühlens and Raven believed they could prove that the ability to hæmolyse varies; this was confirmed by other investigations e. g. Neufeld and Händel in 1907 and Huntemüller in 1911. Huntemüller further maintained that vibrios recently isolated from cases of cholera might also be hæmolytic. All these investigators however used blood-agar, which easily leads to misinterpretations. Sheep's or goat's blood was added, which as later investigations have shown, are very suitable for this purpose; but as van Loghem in a series of publications specially points out, blood-agar is not a suitable medium. He showed that the pale zone which is formed round nearly all vibrio colonies on blood-agar is quite different from the zone formed round the disputed El Tor strains and also from that round most of the cholera-like vibrios.

By spectroscopic examinations of the zone round the El Tor strains he found that the absorption spectrum of oxy-hæmoglobin was intact, contrary to what is the case in the pale zone of the "ordinary" cholera vibrios. This is supposed to be due to the fact that the "ordinary" cholera vibrios during growth digest the hæmoglobin very actively (hæmodigestion) contrary to the El Tor strains which only possessed this power to a slight degree; but, on the other hand, the latter were able to free the hæmoglobin from the red corpuscles, the hæmoglobin then diffusing into the agar and causing the pale zone. In view of these circumstances v. Loghem was against the use of blood-agar for investigations of hæmolysis, and advised the use of fluid media in its place, as in these hæmodigestion does not lead to errors.

v. Loghem believes that true cholera vibrios are not able to produce hæmolysin. If during its growth a vibrio causes true hæmo-

lysis, then, in his opinion, it is not a true cholera vibrio, regardless of its agglutination.

van Loghem's work did not end the controversy regarding the interpretation of the hæmolysis, but caused the technique of these investigations to become more uniform. If it had turned out that only the El Tor strains or strains isolated under similar conditions produced hæmolysis when examined in fluid media, the alterations in the technique would have simplified the problem to some extent, but unfortunately older cholera strains still showed hæmolysis with this technique.

In 1914 Greig examined 333 recently isolated strains of cholera or strains that were less than one year old without being able to find hæmolysin in bouillon cultures, whilst the greater part of 100 other vibrios were hæmolytic.

In the following years the problem lost interest, until Doorenboos in 1930-1931 at El Tor again found hæmolytic vibrios agglutinable with cholera serum, at a period when the epidemiological conditions did not favour the presence of cholera vibrios

Author's investigations:

As a result of the findings described above of the difference between blood-agar and fluid media, I carried out systematic examinations in fluid media only. The technique used is very similar to that described by Greig (1914) except that I used the culture itself and not a filtrate, and that I examined one volume of culture and did not titrate it.

In order to learn the rôle played by the species of animal from which the blood is taken, the first 100 strains or so were examined with red blood corpuscles from the sheep, goat, horse, rabbit and man. Moreover the cultures were examined after 24 hours', 3 and 7 days' incubation at 37 ° C..

Blood recently obtained and to which sodium citrate (0.3 per cent.) had been added, was centrifuged. The plasma was removed and the red blood corpuscles emulsified in 0.9 per cent sodium chloride; they were again centrifuged and emulsified in a fresh solution of sodium chloride. This was repeated until the liquid after centrifuging was clear. A 5 volume per cent. emulsion in solution of so-

dium chloride was then made. Equal parts of red blood corpuscle emulsion and bacterial culture were mixed in a small test tube and incubated at 37° C. for two hours, and the tubes were shaken after one and two hours. The tubes were then placed in the ice-chest at 4° C. and read the next day.

Reading: 0 indicates the tubes in which the fluid was perfectly clear.

1 indicates a slight red zone above the red corpuscles at the bottom of the tubes.

2 indicates a pale, uniform colouring of the fluid, and

3 indicates a strong red colour of the fluid.

It must be mentioned that not all the corpuscles are necessarily dissolved in order to produce the reaction marked 3.

Table 7 gives the results of the investigations with the 100 strains referred to using 5 different sorts of corpuscles and cultures 24 hours, 3 and 7 days old.

Table 7, see p. 82—86.

Table 7.

Test of hæmolysis with erythrocytes from sheep, goat, horse, rabbit and man and cultures 1, 3 and 7 days old.

24 hours' cultures:

Strain No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Sheep	0	0	0	0	0	0	(1)	0	0	0	0	0	0	0	0	0	0	0	(1)		3	0	0	0
Goat	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0
Horse	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		3	0	0	0
Rabbit	3	1	1	3	2	2	3	2	2	3	2	3	3	3	2	2	3	2	3		3	3	2	0
Man	1	0	0	1	(1)	(1)	3	(1)	(1)	2	1	1	1	1	(1)	(1)	1	(1)	2		1	1	(1)	0

72 hours' cultures:

Strain No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Sheep	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	(1)		3	0	0	0
Goat	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		3	0	0	0
Horse	2	1	0	3	0	1	3	1	1	1	2	1	1	1	0	1	1	0	2		3	1	0	0
Rabbit	3	3	3	3	3	3	3	3	3	3	3	3	3	3	2	3	3	3	3		3	3	3	0
Man	3	2	2	3	1	1	3	2	2	3	3	3	3	1	1	3	1	2	3		3	2	1	0

7 days' cultures:

Strain No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Sheep	0	0	0	0	0	0	0	0	0	0	(1)	0	0	0	0	0	0	0	0		3	0	0	0
Goat	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		3	0	0	0
Horse	1	1	1	1	2	1	1	1	2	(1)	3	1	0	1	1	1	1	1	1		3	2	1	(1)
Rabbit	3	3	2	3	3	3	3	3	3	3	3	3	2	3	3	3	3	3	3		3	3	3	0
Man	3	2	1	3	3	3	3	2	2	2	3	3	1	1	1	3	3	2	2		3	3	3	0

24 hours' cultures:

Strain No.	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46
Sheep	0	0	0	3	3	3	0	0	0	0	0	0	0	0	0	3	0	0	0	3	3	0
Goat	0	0	0	3	3	3	0	0	0	0	0	0	0	0	0	0	0	0	0	3	3	0
Horse	0	0	0	2	3	3	0	0	0	0	0	2	0	0	0	3	0	0	0	3	3	1
Rabbit	3	2	3	3	3	3	3	3	3	3	(1)	3	3	3	3	3	3	3	3	3	3	3
Man	0	1	2	2	(1)	2	1	(1)	2	(1)	0	3	1	(1)	2	2	(1)	1	1	3	1	2

72 hours' cultures:

Strain No.	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46
Sheep	0	3	0	3	3	2	0	0	1	0	3	0	0	0	0	2	0	0	0	3	3	0
Goat	0	3	0	3	3	2	0	0	0	0	0	0	0	0	0	2	0	0	0	3	3	0
Horse	1	3	1	3	3	2	0	1	3	1	3	3	2	1	0	3	1	1	1	3	3	1
Rabbit	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
Man	2	3	2	3	3	2	2	2	3	2	0	3	3	3	2	3	3	3	3	3	3	3

7 days' cultures:

Strain No.	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46
Sheep	0	3	0	3	3	0	0	0	2	0	2	0	0	0	0	2	0	0		3	3	0
Goat	3	3	0	3	3	0	0	0	0	0	0	0	0	0	0	1	0	0		3	3	0
Horse	1	3	1	2	3	1	(1)	0	2	(1)	3	2	1	1	1	2	1	1		3	3	1
Rabbit	2	3	2	3	3	2	1	3	3	3	3	3	3	3	3	3	3	3		3	3	3
Man	1	2	1	1	3	0	1	3	3	1	(1)	3	3	3	1	1	3	3		3	3	2

(Table 7 cont.)

24 hours' cultures:

Strain No.	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	64	65	66	67	68	69	70
Sheep	0	0	3	0	0	0	0	1	0	0	0	0	0	3	3	0	0	0	0	0	3	3	3
Goat	0	0	3	0	0	0	0	(1)	0	0	0	0	0	3	3	0	0	0	0	0	3	3	3
Horse	(1)	0	3	0	0	0	0	1	0	0	0	0	0	3	0	0	0	0	0	0	3	3	3
Rabbit	3	2	3	3	3	3	3	2	1	3	2	1	2	3	3	2	3	3	2	2	3	3	3
Man	1	1	(1)	1	1	1	1	0	1	1	1	1	0	2	0	1	2	1	(1)	(1)	2	3	3

72 hours' cultures:

Strain No.	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	64	65	66	67	68	69	70
Sheep	0	0	3	0	0	1	0	3	0	0	0	0	3	3	3	0	0	0	0	0	3	3	3
Goat	0	0	3	0	0	0	0	3	0	0	0	0	3	3	3	0	0	0	0	0	3	3	2
Horse	1	0	3	0	0	1	0	3	1	0	0	0	3	3	(1)	3	3	0	0	0	3	3	3
Rabbit	3	2	3	3	2	3	2	3	3	3	2	1	3	3	3	3	3	2	1	2	3	3	3
Man	2	1	3	1	1	3	1	2	3	2	2	1	1	3	(1)	3	3	2	0	2	3	3	2

7 days' cultures:

Strain No.	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	64	65	66	67	68	69	70
Sheep	0	0	3	0	0	0	0	3	0	0	0	0	0	3	3	0		0	0	0	3	3	0
Goat	0	0	3	0	0	0	0	3	0	0	0	0	0	3	3	0		0	0	0	3	2	0
Horse	1	1	3	1	1	1	1	3	0	0	0	0	1	3	2	1		0	(1)	(1)	3	2	1
Rabbit	3	0	3	2	1	3	(1)	3	3	3	3	2	3	3	3	2		3	1	3	3	3	2
Man	2	0	3	1	0	3	(1)	2	2	2	2	1	1	3	1	2		2	1	3	3	1	1

24 hours' cultures:

Strain No.	71	72	73	74	75	76	78	79	80	81	82	83	84	85	86	87	88	89	90	91	93	94
Sheep	3	0	0	0	0	0	0	3	0	3	0	0	2	3	0	0	2		0	0	3	3
Goat	3	0	0	0	0	0	0	1	0	3	0	0	2	3	0	0	(1)		0	0	3	3
Horse	3	0	0	0	0	0	0	1	1	3	0	0	1	3	0	0	(1)		0	0	3	3
Rabbit	3	3	3	3	2	3	0	3	3	3	1	0	2	3	(1)	2	2		1	2	3	3
Man	3	3	1	1	0	1	0	1	3	0	0	0	0	1	0	(1)	1		0	0	3	3

72 hours' cultures:

Strain No.	71	72	73	74	75	76	78	79	80	81	82	83	84	85	86	87	88	89	90	91	93	94
Sheep	3	0	0	0	0	0	0	2	0	3	3	0	3	3	2	0	1	0	0	0	3	3
Goat	3	0	0	0	0	0	0	0	0	3	3	0	3	3	2	0	0	0	0	0	3	3
Horse	3	0	0	(1)	(1)	0	0	(1)	2	3	3	0	3	3	3	(1)	(1)	(1)	0	3	3	3
Rabbit	3	2	3	3	3	2	0	3	3	3	3	0	3	3	3	3	3	3	3	3	3	3
Man	3	0	3	2	2	2	0	1	2	1	2	0	(1)	1	0	3	1	1	2	3	3	3

7 days' cultures:

Strain No.	71	72	73	74	75	76	78	79	80	81	82	83	84	85	86	87	88	89	90	91	93	94
Sheep	3	0	0	0	0		0	2	0	0	3	0	3	3	2	0	2	3	0	0	(1)	3
Goat	3	0	0	0	0		0	1	0	0	3	0	3	3	2	0	1	3	0	0	(1)	3
Horse	3	1	1	0	1		0	2	1	0	3	0	3	3	3	(1)	2	3	(1)	1	2	3
Rabbit	3	2	2	2	3		0	3	3	3	3	0	3	3	3	3	3	3	3	2	3	3
Man	3	1	1	2	2		0	1	2	1	3	0	3	3	0	2	(1)	1	3	1	1	3

(Table 7 cont.)

24 hours' cultures:

Strain No.	95	96	97	98	99	100	101	102	103	104	105
Sheep	0	0	0	0	0	(1)	3		3	3	3
Goat	0	0	0	0	0	(1)	3		3	3	3
Horse	0	0	0	0	0	(1)	3		3	3	3
Rabbit	2	3	2	3	3	3	3		3	3	3
Man	1	1	0	1	1	(1)	3		3	3	3

72 hours' cultures:

Strain No.	95	96	97	98	99	100	101	102	103	104	105
Sheep	0	0	0	0	0	3	3	3	3	3	3
Goat	0	0	0	0	0	3	3	3	3	3	3
Horse	1	1	(1)	(1)	(1)	3	3	3	3	3	3
Rabbit	3	3	3	3	3	3	3	3	3	3	3
Man	1	3	3	3	3	3	3	3	3	3	3

7 days' cultures:

Strain No.	95	96	97	98	99	100	101	102	103	104	105
Sheep	0	0	(1)	0	0	3	3	1	3		3
Goat	0	0	0	0	0	3	3	2	3		3
Horse	(1)	1	3	0	0	3	3	2	3		3
Rabbit	(1)	2	3	0	(1)	3	3	3	3		3
Man	(1)	2	3	0	(1)	3	3	0	3		0

From the table it will be seen that a few of the strains do not hæmolyse any of the corpuscles at all. Exceptions made of these, the general results are as follows:

Rabbit's blood is hæmolysed strongly by nearly all the strains, after 24 hours, 3 or 7 days. Three of the strains, however — 31, 51 and 53 — showed slighter hæmolysis after 7 days than after 24 hours or 3 days.

Most of the 24 hours' strains hæmolysed human corpuscles to some degree, hæmolysis often increasing to a fair extent after 3 and 7 days. Only 4 strains hæmolysed human corpuscles very slightly or not at all.

24 hours' cultures did not generally hæmolyse horse blood corpuscles, except in the cases in which all the corpuscles investigated were hæmolysed. The 3 or 7 days' old cultures, on the other hand, generally did show hæmolysis, varying from slight to strong.

Sheep's blood was hæmolysed only in a few cases, except when all corpuscles were hæmolysed. Exceptions were strains 33 and 35 in 3 and 7 days, 40 in 24 hours and 52 in 3 days old cultures.

Goat's blood corpuscles were the least sensitive. They were only hæmolysed in the cases in which all kinds of corpuscles were hæmolysed. When these corpuscles were hæmolysed strong hæmolysis generally occurred.

Cultures of 6 of the strains which hæmolysed goat's blood after 24 hours and after 3 days, turned out on repeated examination to be unable to hæmolyse after the 7th day. This shows the slight stability of these hæmolysins.

In all the experiments on hæmolysis, the stability of the blood corpuscle emulsion was tested with a control tube containing the emulsion with the addition of sterile bouillon instead of culture.

Basing himself on the experience gained in the above investigation, the author examined the remainder of the strains in 3 days' cultures towards goat's blood. But some of the strains examined previously now gave varying results. Strains which at the first examination had not hæmolysed at all were now strongly hæmolytic and vice versa. These variations, however, only occurred amongst the strains that were inagglutinable with cholera serum.

As these variations were accidentally observed several times, a number of strains were examined at different times as to their hæmolytic power. The results of this investigation is shown in Table 8.

Table 8.

The hæmolytic power by the same strains at different times.

Strain No.	21	24	28	29	33	35	39	40	60	61	68	69	70	72	73	75	76
Febr. 1933.	3	0	3	3	0	0	0	2	3	3	3	3	2	0	0	0	0
Beg. Sept. 33.	3	0	3	3	3	3	3	3	3	1	3	2	3	0	0	0	0
End Sept. 33.						3	(1)								0	3	

Strain No.	78	79	81	82	83	84	85	86	88	93	102	104	105	109
Febr. 1933.	0	0	3	3	0	3	3	2	0	3	3	3	3	3
Beg. Sept. 33.	0	3	1	0	0	3	3	3	3	3	3	3	3	3
End Sept. 33.		3		(1)					3					

Strain No.	112	159	167	182	204	240	251	259	282	284	285		286	291	292	294
June 1933.	3	1	0	3	3	0	0	0	0	3	3	July 1933	0	3	3	3
Beg. Sept. 33.	3	3	0	3	3	0	0	0	0	3	3		0	3	3	3
End Sept. 33.		3						0	0				0			

Strain No.	295	296	297	298	299	300	301	302	303	304	305	306	307	308	309	310	311
July 1933.	3	1	0	3	0	0	2	0	2	3	0	3	3	2	3	3	0
Aug.-Sept. 1933.	3	3	0	3	3	3	3	3	3	3	0	3	3	3	3	3	(1)
End Sept. 33.					3	3		3									0

Strain No.	313	314	315	316	317	318	319	320	321	323	324	325	326	327	328	329	330
July 1933.	2	0	2	1	1	0	2	2	2	3	3	0	(1)	(1)	(1)	3	3
Aug.-Sept. 1933.	3	0	3	2	3	0	3	3	3	3	3	1	3	3	3	3	3
End Sept. 33.		0										1	3	3	3		

Strain No.	331	333	334	335	336	337	338	339	340	341	343	345	346	347	348	349	350
July 1933.	3	3	3	3	0	3	3	0	3	3	0	3	3	3	3	0	3
Aug.-Sept. 1933.	3	3	3	3	0	3	3	0	3	3	3	3	3	3	0	0	3
End Sept. 33.					3			0							0		

Strain No.	351	354	355	356	357			1	2	3	4	19	20	22	44	45	48
July 1933.	3	3	3	3	3	March 1933		0	0	0	0	0	0	0	3	3	0
Aug.-Sept. 1933.	3	3	3	3	3	End Sept. 1933		0	0	0	0	0	0	0	3	3	0

Strain No.	49	50	54	71	94	101	103	106	110	113	114	115	258	322	369	Contr.
Febr.-March 1933.	3	0	3	3	3	3	3	3	0	3	0	0	3	0	3	0
End Sept. 33.	3	0	3	3	3	3	3	3	0	3	0	0	0	0	3	0

This table clearly shows that the formation of substances which, with the technique used here, cause hæmolysis of the blood corpuscles, is not a constant phenomenon. Thus the strains 33, 35, 39, 79, 88, 159, 296, 299, 300, 302, 317, 326, 327, 328, 336 and 343 did not show any hæmolysis in the first tests, contrary to what happened in later experiments, in which their hæmolytic power was strongly developed; the strains 61, 81 and 258 showed exactly the opposite phenomenons.

In order to gain, if possible, more knowledge of the conditions governing these variations, the hæmolytic power of bouillon cultures spread from numerous single colonies on the same agar plate of the same strain were examined.

For the experiment 4 cholera-like strains which had shown variations in the preliminary experiments, were chosen, namely 33, 35, 39 and 282, and in addition 4 strains that were agglutinable with cholera serum, namely 2 strains 45 and 54, which had shown hæmolytic stability, and 2 strains 34 and 90, that had hitherto been non-hæmolytic.

24 hours' bouillon cultures were spread on agar; after 20 hours' incubation at 37° C. bouillon cultures were made from 20 separate colonies, and these cultures were incubated at 37° C. for 3 days. The colonies in 6 cases were chosen at random, as they were all alike. 2 of the strains, 39 and 282, showed morphologically different types of colonies; in addition to the normal type, strain 39, showed some more domed and dense colonies, and strain 282 some that were wrinkled. In these cases 10 normal colonies and 10 of the variations were chosen.

The results of these experiments with 3 days' bouillon cultures are indicated in Table 9.

These experiments already show that the laws regulating the formation of hæmolytic substances are apparently not quite simple, colonies from the same inoculation behaving quite differently. From a few of the cultures examined in this experiment, a new inoculation was made on agar and 10 separate colonies from each plate were then examined.

After these experiments had been read — the results are given in Table 10 —, some of these cultures were again inoculated, and 5 separate colonies examined. The results are given in Table 11.

Table 9.

Examinations of hæmolysis in bouillon cultures from single colonies on an agar plate.

	a	b	c	d	e	f	g	h	i	k	l	m	n	o	p	r	s	t	u	v
Str. 33.	3	3	3	1	3	3	2	1	3	3	(1)	3	2	3	2	0*)	1	1	2	2
— 35.	0	(1)	(1)	0	2	3	0	0	3	0	0	(1)	3	1	3	0	0	3	0	1
— 39.	1	0	3	1	3	3	(1)	1	0	3	3	3	3	3	3	0	3	3	3	3
— 282.	2	3	1	2	0	1	0	1	1	0	2	2	2	3	3	1	3	1	0	1
— 45.	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
— 54.	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
— 34.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
— 90.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

*) The culture produced only a floccular precipitate.

Table 10.

Examinations of hæmolysis in bouillon cultures from a single colony on an agar plate, spread from the cultures examined in Table 9.

				1	2	3	4	5	6	7	8	9	10	
Strain No. 33 a-parent culture	strongly	hæmolytic.		2	3	3	0	3	0	3	0	0	3	control:
— - 33 l- —	very weak	—		3	3	3	3	3	3	3	3	3	3	0
— - 35 a- —	not	—		1	0	1	3	0	3	0	2	1	2	
— - 35 f- —	strongly	—		3	3	3	3	3	3	3	3	3	3	
— - 39 c- —	—	—		3	3	3	3	3	3	3	3	3	3	
— - 39 i- —	not	—		3	0	3	0	0	1	3	3	1	0	
— - 282 e- —	—	—		0	(1)	1	0	0	0	1	(1)	0	0	
— - 282 p- —	strongly	—		0	(1)	0	1	0	0	0	0	0	0	

Table 11.

Examinations of cultures from single colonies of some of the strains in Table 10.

Strain	No.						
Strain	No.	33 a_2.	3	3	3	3	3
—	-	33 a_4.	3	3	3	3	3
—	-	33 l_1.	3	3	3	3	3
—	-	35 a_2.	(1)	0	(1)	(1)	1
—	-	35 a_4.	2	2	2	2	2
—	-	35 f_1.	2	1	2	2	2
—	-	39 c_1.	3	3	3	3	3
—	-	39 i_1.	3	3	3	3	3
—	-	39 i_2.	0	0	0	0	0
—	-	282 e_1.	0	1	(1)	0	1
—	-	282 e_8.	1	(1)	0	(1)	1
—	-	282 p_1.	0	0	0	0	0
—	-	282 p_4.	(1)	(1)	(1)	0	1

From these experiments with cultures taken from the same inoculation, and from 3 successive transfers it may be seen, that the power of hæmolysis is hardly related to simple exogenous or hereditary factors, but that it seems to depend on laws which only more complicated investigations may be able to reveal; but although the cause of the variability is still unknown, the experiments show that a grouping of the vibrios based on their hæmolytic power as determined with the technique described here, is not reliable.

As Doorenboos (1932) stated that the presence of bacteriophage may interfere with these phenomena, these 8 strains were examined for "mixture" of bacteriophage.

The investigation was carried out as follows: A "young" — 5 hours' — bouillon culture was mixed with an "older" — 72 hours' — bouillon culture, and the mixture filtered through a Chamberlain filter L_3. A drop of the filtrate was poured in a line on an agar plate, freshly spread with the homologous strain. The plates were examined after 20—24 hours' incubation at 37 ° C.. None of the plates showed any sign of the effects of bacteriophage following the line. On the plate spread with strain 39 there was everywhere, and not only localized to the line on which the filtrate

had been poured, numerous dots of an appearance very reminiscent of plaques of phage. It is true that these negative results do not exclude the possibility that the cultures were "infected" with phage; for if it were a question of strains resistant to phage, they would be very unsuitable for the demonstration of "plaques"; but if this were the case, practically all the elements should have become resistant, and it is very unlikely that a perfectly resistant culture would have conveyed the bacteriophage through numerous subinoculations.

Although the power of hæmolysis varies, de Vogel's report that amongst more than 400 freshly isolated cholera strains, none showed power of hæmolysis, is nevertheless of great interest.

Investigations into this problem in the future must be carried out with freshly isolated strains and with as reliable a technique as possible.

Chapter VI.

SEROLOGICAL INVESTIGATIONS

a) Survey of serological literature.
b) Author's investigations. Technique.
c) Investigations on Agglutination.
d) Investigations on 0 and H antigen.
e) Absorption experiments.

a) *Survey of serological literature.*

Numerous bacteria may be identified by their biochemical, cultural and morphological characters; but in regard to the vibrios this was previously not the case, and it was generally necessary to seek the help of serological examinations in order to determine whether a vibrio was V. Choleræ or a cholera-like vibrio.

The first serological reaction used for the identification of a vibrio as a cholera vibrio, was the bacteriolytic reaction, as it was observed by Pfeiffer and described by him together with Issaeff in several publications from 1894—95. After intraperitoneal injection of vibrios in a guinea pig either previously immunized with a homologous strain or simultaneously injected intraperitoneally with a homologous serum, Pfeiffer found that the injected vibrios were dissolved in ½—2 hours. If the animal had not been treated or had been immunized with a strain that was non-homologous, or if the serum used was not homologous, the bacteriolysis did not take place. The reaction, which is called Pfeiffer's phenomenon, was in practical diagnostic work soon succeeded by the much simpler agglutination test, after it had been shown that the results of the latter practically speaking always agreed with those of the former. At present the Pfeiffer phenomenon is chiefly of historical interest.

It has already been mentioned, that when the agglutination test was first introduced, the cholera vibrio amongst other bacteria was used for the experiments described.

In their first papers on agglutination Gruber and Durham in 1896 described the reaction as they had observed it macroscopically in small test-tubes and microscopically on slides, on which were placed a culture together with the serum from an animal treated beforehand with bacilli of the species in question.

As described in the introduction, agglutination was immediately introduced in practical diagnostic work, but the finding of the agglutinable vibrios at El Tor in 1905 under epidemiological conditions, which according to some investigators made it very unlikely that they were true cholera vibrios, caused doubt as to the specificity of the reaction in this group of bacteria.

In order to investigate whether methods of examination other than that of agglutination could prove any differences between the antigenic composition of true cholera vibrios and the El Tor strains, Neufeld and Händel attempted experiments with the complement-fixation test. They found that the antigen of the El Tor strains fixed the antibodies of cholera serum to the same degree as true cholera strains. Moreover they found that in several cases the complement-fixation test, with their technique at any rate, did not give as clear results in distinguishing between cholera vibrios and cholera-like vibrios as the agglutination test and the Pfeiffer phenomenon. It is necessary to mention that Neufeld and Händel did not carry out either cross absorption experiments or cross complement-fixation tests.

Besides the question of the comparison of the antigenic composition of the El Tor strains and the V. Choleræ two large problems in connection with the serological methods remained to be examined. One was whether the vibrios agglutinable with cholera serum included several serologically different types; the other was to find a classification of the vibrios inagglutinable with cholera serum, and possibly elucidate their pathogenecity in this connection.

In the paper of Kolle and Gotschlich and their collaborators published in 1903 it is stated that, in their opinion, asiatic cholera can only be produced by a single species of vibrios. This was, however, more a supposition than a statement based on experiments, but it agreed with the prevailing views; and for many years it was the general opinion that this was the case, and nobody attempted to find a systematic solution of the problem as to whether the cholera vibrios

really constituted a homologous species of vibrios. Not until 1921 was the question examined experimentally by Douglas.

He tested 9 cholera strains, isolated at different places, and from 3 of these strains he produced sera. Each of the strains were examined with the agglutination test against these 3 sera, and he found that they were agglutinated almost equally by all three. Absorption of one serum by 5 of the strains showed that they were all able to remove all agglutinin from the serum. From this he concluded that at any rate amongst the strains of cholera examined in the experiment quoted no difference in antigens existed. In addition to these commonly accepted cholera vibrios, he also examined some El Tor strains and other vibrios; by absorption with several of the El Tor strains he found that they were able to remove all the agglutinin from a cholera serum, and he concluded that the El Tor strains must be true cholera vibrios, a conclusion that was not justified on the basis of the experiments carried out. For he omitted to examine whether cholera vibrios are able to remove all agglutinin from El Tor serum. The fact that in order to prove the identity of 2 strains it is insufficient to carry out an absorption test of one serum with both strains, but both sera must be absorbed by both strains has been emphasized by Oluf Thomsen (1924) and later by Jessen (1934) in his investigations on types of meningococci.

For his absorption experiments Douglas used formolized bouillon culture as antigen; the results of this technique, however, are not always analogous to the results obtained with live bacterial emulsions in saline solution.

As a contribution to the question whether there are several serologically different types of cholera vibrios, Kabeshima in 1918 published in French some experiments with 2 different vibrios, previously published in Japanese; one of these vibrios, J, had been isolated during a cholera epidemic, the other, F, from a sporadic case of diarrhoea resembling cholera. The latter fact shows the weakness of the statement that the second is a true cholera vibrio. Kabeshima found that each of the two vibrios were only slightly agglutinated by the serum of the other, and cross-absorption experiments showed that they couldn't absorb much agglutinin from the heterologous serum. Kabeshima considered that he had found a

link between J and F by cultivating F in bouillon to which F serum had been added, an opinion which may now possibly be explained by our knowledge of transitions to spontaneously agglutinable, rough forms of bacteria cultivated in media to which homologous serum has been added. Japanese investigators later called the two types J and F »original« and »varié«.

Kabeshima's investigations were carried on by Nobechi, who believed that there was still one type »intermédiaire« which, however, only differed slightly from »original«.

From his absorption experiments Nobechi drew the conclusion that the antigenic composition of the three types might schematically be expressed as follows: »original« AX, »intermédiaire« A(B)X and »varié« BX, X representing the non-specific group factor.

Nobechi's absorption table is given in Table 12:

Table 12.

serum	strain examined	titre before absorption	titre after absorption with original	intermédiaire	varié
original AX	original	25600	0	0	3200
	intermédiaire	25600	0	0	1600
	varié	25600	0	0	0
intermédiaire A(B)X	original	12800	0	0	6400
	intermédiaire	12800	800	0	6400
	varié	12800	800	0	0
varié BX	original	800	0	0	0
	intermédiaire	3200	800	0	0
	varié	6400	1600	1600	0

If this antigen table were correct, the serum of "varié" should be quite exhausted by the strain "intermédiaire", but this is not the case. Nobechi might more correctly have erpressed the scheme as AX, ABX, and BCX, introducing a fourth factor C in "varié".

Two other Japanese authors Inouye and Kakihara, in a paper published in 1925, thought they could confirm Nobechi's assertion of three types, but they suggested that these should be called type I (original), type II (varié) and type III (intermédiaire).

The three types of Nobechi have been used as a basis for the examination of strains isolated during a cholera epidemic at Shanghai in 1932. The investigation has been published by three Japanese authors, a temporary report by Kuroya and Oshio, and a larger final report by Kuroya and Ōno. This work was carried out at a Japanese Institute in Shanghai.

In both papers absorption experiments are described. These showed that amongst a total of 68 strains isolated during the whole of the epidemic, 60 were type varié, and 8 appeared to be type intermédiaire. An examination of the epidemiological data compared with the bacteriological findings is of no significance, as the material is too small in relation to the size of the area from which it has been collected.

If Kabeshima's agglutination results are compared with Nobechi's table of experiments, it is seen that these two investigators reach somewhat divergent results in regard to the quantitive agglutination of type original and type varié.

Scholten of Leyden showed by absorption experiments in 1933 that amongst 8 strains, agglutinable with cholera serum, there were two types with an antigenic composition which he calls A and AB. This finding agrees perfectly with my results. It so happens that 2 of the strains examined by Scholten are even included in my material. The technique used by Scholten is in the main similar to that in the present experiments.

Apart from the work by the authors mentioned here, the question of several types of vibrios with a mutual antigenic factor has not been more closely examined.

The possible existence of O and H factors and their importance in a classification of the vibrios will be discussed in the chapter on O and H antigens.

Two Japanese, Aoki and Oshiro wrote a paper in 1934 in which thay state that they found the three types of Nobechi representing a specific form, a non-specific form and a mixed form, these terms being used as within the group of salmonella; but Aoki and Oshiro state that the specifics forms are thermostable, and the non-specific partly thermostable and partly thermolabile. On the whole this paper is merely a repetition of the previous Japanese publications on the three types.

The other problem, the attempt to classify the vibrios inagglutinable with cholera serum and the examination of their pathogenecity in this connection, was taken up by Greig in 1914 and the following years. He carried out his investigations in the North of India, where in a material of 659 clinical cases of cholera he isolated cholera vibrios alone in 77 per cent. of the cases, in 10,5 per cent. he did not find any vibrios at all, in 8 per cent. he found cholera-like vibrios, and in 4—5 per cent he found cholera vibrios and cholera-like vibrios together.

The fairly frequent occurrence of the cholera-like vibrios in these cases — cholera-like meaning vibrios inagglutinable with cholera serum — is of some interest. The question at once arises as to whether they are cholera vibrios, occurring in an inagglutinable phase. For this reason, amongst others, Greig investigated whether it was possible to agglutinate them with other sera, and when this occurred, whether it was possible to classify them on the basis of their agglutination. In this way he found that amongst 75 cholera-like vibrios, it was possible to classify 62 in 9 groups, each including 2—30 specimens, whilst 13 could not be grouped at all.

Thus, there is no reason to assume that cholera vibrios often occur in an inagglutinable phase.

Greig deals with the pathogenecity of these vibrios only very briefly; in several cases he examined convalescent serum as to its content of agglutinin towards the vibrio isolated from the patient. Most frequently he did not find any agglutinin in the serum at all, in a few cases agglutinin was found in very feeble concentration. These findings give us no information about the pathogenecity of the vibrios. Greig believes, however, that cases clinically resembling cholera may be produced by vibrios other than the true cholera vibrio. In support of this statement he states that in some of the cases in which vibrios belonging to his numerically largest group had been isolated alone the course of the disease was far milder than in the cases in which the true Vibrio Choleræ had been isolated simultaneously. On the other hand, the large number of groups containing only one or two microbes indicates that some of them are non-pathogenetic; were the opposite the case, smaller epidemics with the more frequent presence of the pathogenetic microbe would be expected to occur under the bad hygiene prevailing there.

Greig did not investigate whether cholera-like vibrios may be isolated from healthy individuals living under the same conditions as the patients.

In another paper Greig (1916) reports the examination of 39 vibrios isolated from wells and rivers. Amongst these he found 6 groups, each containing 3—6 strains, whilst 7 strains could not be grouped.

Chalmers and Waterfield (1916) who in 1915 had isolated a cholera-like vibrio from a case of acute diarrhoea, in their report on this case made some remarks on the grouping of vibrios. They named 7 different groups, using the following as criteria for their classification: growth in phosphorescent or non-phosphorescent cultures, gelatine liquefaction, power of pigment formation in peptone-water, the relationship towards cholera serum, the appearance of gelatine stab cultures and lastly, possible pathogenecity towards doves. In the opinion of these authors each of these delimited a separate group.

Considering the importance which bacteriologists attach nowadays to the antigenic conditions and partly also to biochemical reactions, Chalmers and Waterfield's "system" becomes of mere historical interest, as one of the first attempts made to group the vibrios according to the conditions mentioned.

In 1922 Mackie stated that he had attempted in Egypt to group 57 cholera-like vibrios according to their agglutination reactions, and had found that these 57 strains, isolated partly from cases of diarrhoea and partly from healthy persons, had to be divided into 20 groups. In a previous paper published in 1918 Mackie and Storer reported the results of bacteriological examinations in a very small epidemic of diarrhoea. Within a very limited area 7 cases of diarrhoea occurred in 13 days. In the stools of 6 of the patients it was possible to find vibrios either by direct microscopical examination or by cultivation. The vibrios disappeared together with the symptoms of the disease. 4 of the strains were agglutinable by one serum, 2 by another. In one case there was a positive sero-reaction against the same strain isolated from the patient. On examining the stools of healthy persons in the patients' environment, vibrios belonging to both types were found. The authors believe that the vibrios they isolated are pathogenetic,

but produce a disease which has not the same epidemiological character as cholera, whilst clinically the symptoms may vary. The cases in which the vibrios had been isolated from healthy persons, they considered to be carriers.

More recently Mackie (2) has reported that both he and others have isolated vibrios agglutinable with sera corresponding to the two strains mentioned, partly from sporadic cases, and partly from very limited epidemics of cholera-like cases.

Mackie reports that examination of a number of healthy Egyptians did not show any vibrios. This indicates that the vibrios isolated in cases of diarrhoea were pathogenetic. The finding of vibrios in the stools of individuals in patients' environment points neither one way nor the other.

The publications dealing with the classifications of cholera-like vibrios quoted up to the present, have all the character of preliminary investigations in regard to the pathogenecity of these groups; none of them give a final view on any problem of the subject. So far we still need work in which the pathogenecity of the isolated vibrios will be proved with the greatest certainty by combined epidemiological, clinical, bacteriological and serological examinations.

The present investigations do not pretend to throw direct light on this problem. The serological classification is intented as a basis for the problem of *serological classification in relation to the biochemical reactions;* but a system of classification based on such reactions forms a necessary laboratory foundation for epidemiological examinations and examinations on pathogenecity.

Some of the results have already been reported in May 1934 (Heiberg (2)).

Whilst the importance of the thermostable antigens belonging to the bacterial body — O antigens — and the thermolabile flagella antigens — H antigens — is commonly accepted in the grouping of the Gram-negative, pathogenic rods, the conditions are different within the group of vibrios.

Weil and Felix, who in an extensive paper published in 1920 surveyed their investigations on the appearance of O and H antigen in different bacteria, state that in preliminary examinations on cholera vibrios they had observed granular agglutination only and

not, as in the salmonella group, both granules and looser flocculation. This finding is supposed to indicate the presence of only one antigen, and if conditions here were analogous to those in the salmonella group, it should indicate that there is only O antigen present.

The results of Weil and Felix were verified by Brutsaert in 1924. By examinations of two strains of V. Choleræ and their corresponding sera by agglutination and absorption he was able to find thermostabile antigen only.

But where these investigators failed — i. e. in proving H antigen — later workers appear to have succeeded.

Thus Balteanu in 1926 wrote that by the agglutination of cholera vibrios he was able to find loose flocculation, corresponding to the reaction between flagella antigen and its agglutinin, and finer granulation, corresponding to the O factor. He used both absorption experiments and examinations with fresh and heated sera, and he found that the behaviour of the antigens and agglutinins towards heat corresponds to what is seen with the paratyphoid bacillus. It must be mentioned that Balteanu used emulsions of agar cultures without the addition of formalin or similar substance, for his agglutination tests. He read the tests after 3 hours incubation at 50° C. and again after 24 and 48 hours in the ice-chest. Moreover, in some of his experiments he used as special H antigen an emulsion of live vibrios from which the bacterial bodies after some hours' shaking were centrifuged.

Balteanu's work was verified in 1931 by the two Egyptians, Gohar and Abdoosh. Both these authors also deal with the possibility of these two antigen factors being group-specific, especially within the cholera-like vibrios. Gohar did not think that either H or O antigen were group specific, but he considered that the O antigen might delimit larger groups of vibrios, and within each of these groups there might be different quantities of H antigen, and vice-verca; the H antigen may delimit larger groups within which there are different quantities of the O factor. Abdoosh, on the other hand, thought that only O antigen might delimit smaller groups, in contrast to the H antigen, which was found in all groups.

Further, Gardner (1934) in 1933 in a report to the "Office International d'Hygiène Publique" published some of his investigations into this problem. In 2 sera produced for general diagnostic work, he found two different agglutinins, which could be separated by absorption with boiled cultures; moreover, by using formolized bouillon cultures for his agglutination experiments, and reading after 24 hours at 52° C. he found reactions reminiscent of H agglutination; these reactions did not appear if boiled cultures were used as antigen. The titre of the two factors was very different in serum, highest for the H factor. With some of the strains he could not produce any reaction at all with boiled cultures.

A discussion in connection with the occurrence of O and H antigen and their importance in practical diagnosis will follow the description of my own experiments.

The variation in agglutinogenic and agglutinative powers within each separate strain of bacteria is a problem of great importance. Acquisition as well as loss of antigenic factors during cultivation in laboratories is a well-known phenomenon as far as other bacterial groups are concerned, and has often been described together with changes in cultural characters.

At present the problem is being examined in regard to the cholera vibrio in several laboratories, and some publications have already appeared on the subject. In 1924 Shousha found that one of his cholera strains grew in morphologically differing colonies. Their appearance was such that he called them rough and smooth, analogous to the terms used in connection with other bacteria. He found that these two forms were identical as regards motility and biochemical reactions, but they showed only slight resemblance in regard to agglutinogenic and agglutinative powers.

In the paper previously mentioned Baltanu decribed (1926) 3 different types of colonies, which he had observed in subcultures on agar from bouillon cultures several weeks old; these three types differed morphologically from the normal type. Two of these forms returned to the normal type of colony on subculture, whilst the third corresponded to a rough type. These colonies were described as round, unusually prominent, opaque, sometimes having a wrink-

led surface. In transmitted light they are dense and white, of firm consistency and they adhere to the surface.

They retained their form of colony, even after animal passage. Their antigen corresponded to the O antigen of the parent strain.

Pasricha, de Monte and Gupta described in 1932 2 types of colonies differing from the normal type, one however, most likely forming the link between the original type and the other. They were colonies of rough appearance, and one type remained the same through several passages. The colonies were emulsified with difficulty in saline and distilled water, but in regard to their biochemical and serological reactions they behaved like true cholera vibrios.

A report from two Indian investigators, Gouyle and Sen Gupta (1932) contradicts the above observations. They found that the antigenic powers of rough and smooth colonies differed. These experiments agree with the results of Yang and Bruce White (1934). But a comparison between different investigations on the properties of rough and smooth strains is very difficult indeed, owing to the lack of criteria as to the terms rough and smooth.

Balteanu and Pasricha emphasized the appearance of the colonies and described the rough colonies as really dry and wrinkled in appearance, Gouyle and Sen Gupta did not find that the colonies resembled the characteristic rough colonies of other bacteria; they took into consideration the power of spontaneous agglutination. Yang and White, on the other hand, emphasizing the reaction of the strain towards Millon's reagent, admitted that even their apparently smoothest colony showed slight flocculation with this reagent.

The last statement indicates either that Millon's reagent is not suitable for determining whether a strain is smooth or rough, or that the vibrios very easily develop rough forms. My own experiments with Millon's reagent have also shown that it was not possible by its help to distinguish two groups of strains, as all the 50 strains examined, including a morphologically perfectly smooth strain of paratyphoid, were flocculated by the reagent.

In the paper by Pasricha and his collaborators already mentioned, they describe another type of variation which they observed in the cholera vibrios. After repeated passages on gall-salt-agar plates they were able to isolate colonies, which had changed their

antigenic powers in such a manner that cross agglutination did not show antigen mutual to the parent strain. They state, that they have previously proved that in the laboratory cholera-like vibrios may become agglutinable with cholera serum.

This phenomenon, that the agglutinating and agglutinogenous powers of vibrios may vary, had also previously been responsible for apparently contradictory reports.

It is also known in regard to other bacteria that agglutinability may be lost and agglutinogenic powers may vary, and it is possible that under certain conditions these powers are particularly labile in the vibrios, but it does not seem likely that this would make it impossible to determine broad lines in the antigen "construction" of the vibrios. As regards some of the antigens at any rate, numerous strains have shown great constancy in the laboratory for 20 to 30 years.

If the vibrios possessed such marked power of changing their reactions, it would be expected that this would cause difficulties in the present work as regards the possibility of forming a classificition based on biochemical reactions; this has, however, not proved the case.

b) *Author's investigations; technique:*

Production of serum. For standard production of serum rabbits were given intravenous injections of a 20 hours' agar culture, emulsified in normal saline in a concentration of about 1000 million vibrios per cc. The culture was killed by heating for 20 minutes at 55 ° C.. The rabbits were generally given 3 injections at intervals of 5 days, and 5 days after the last injection they were completely bled by severing the throat vessels. After the coagulum had been removed from the clotted blood, and the remaining erythrocytes removed by centrifuging, tricresol (in ether) was added, giving a concentration of ⅓ per thousand.

The letter S in front of a figure indicates a serum produced from the strain bearing this number in my material.

Agglutination. As the standard method I used for antigen an emulsion of a 20—24 hours' agar culture — pH of the agar about 7,6 — the emulsion in normal saline containing about 1000 million vibrios per cc. The dilutions of serum were made with normal saline.

0,3 cc culture was added to 0,2 cc dilution of serum, giving a

total volume of 0,5 cc. The dilution of the serum indicates the dilution in the total volume of 0,5 cc in the Widal tube. The tubes used were round-bottomed. The agglutinations were carried out in 5 serum dilutions, the dilution in the last tube corresponding to the titre T of the homologous strain. In the first tube the dilution was T/16 and in the 3 middle tubes T/8, T/4 and T/2.

After shaking the tubes were incubated at 37 ° C. for two hours and then left at room temperature for 16—18 hours before they were read. Sometimes the reactions were read at 2 hours. This did not show large variations from the readings on the following day, except that the late reading caused the reaction to include one more tube. Similarly incubation at 37 ° C. overnight did not influence the results to a degree of any importance. The readings were made without using a magnifying glass.

In the tables, "3" indicates strong agglutination and supernatant fluid clear, "2" a great deposit at the bottom of the tube, but supernatant fluid not quite clear, "1" only weak agglutination and "0" no agglutination at all.

As discussed on p. 143 agglutination with this technique is due to the reaction of a thermostable antigen. When another technique is used — see p. 153 — quite different reactions may be produced.

Agglutination on microscopic slide. Besides the technique described above, agglutination has in some cases first been carried out on a microscopic slide. The method used was as follows:

2—3 drops are placed on a slide, and with a little practice it is possible to judge the size of the drops. They must be so small that a rocking movement of the slide does not cause them to merge, and so large that they allow the formation of currents when the slide is rocked.

The first drop placed on the slide is saline, the next one or two the serum to be examined. The dilution of the serum is determined empirically from the homologous strain, the dilution used being one that causes immediate macroscopically visible agglutination of the emulsified bacteria — i. e. within 5—10 seconds. As the reaction must appear quickly, it is necessary to use fairly high concentrations of serum, e. g. 1/10—1/100, according to the strength of the serum. In this serum a quantity of 20—24 hours' agar culture is suspended, sufficient to make the drop opaque, but not to make it impossible to

distinguish the particles that may drop out during agglutination; the emulsion is made as homogenous as possible by carefully stirring the culture into the serum.

After emulsification the slide is rocked between the fingers 5—8 times. The reading is taken most easily if the slide is held obliquely, the greater part of the liquid lying at the lower end of the »lake«; it will then be simple to see whether there are agglutinated particles or whether the liquid still has a milky appearance at the upper end of the »lake«.

Absorption experiments. The absorption of agglutinins from serum has been carried out in the following manner: a very concentrated emulsion of 18—20 hours' agar culture was heated for 20 minutes at 55 ° C. and was then mixed with equal parts of serum in a dilution corresponding to 1/80 of the titre. This made the dilution of serum 1/40 of the titre. After shaking this mixture was incubated at 37 ° C. for 2 hours; then it was again shaken and left at room temperature for 4—6 hours or until the next day; i. e. 16—20 hours. The bacterial bodies with possible absorbed agglutinin were removed by strong centrifuging, i. e. until the liquid above was practically clear. The liquid was now ready for use, its dilution just corresponding to the dilution to be used for the first tube. In this tube the dilution should be 1/16; mixture of 0.2 cc serum dilution and 0.3 cc culture make a further dilution of serum: $\frac{T}{40} \times 2.5$ equals T/16.

The agglutination reactions were carried out as described p. 105.

The abbreviations, etc. used in the tables are explained p. 154.

c) *Investigations on Agglutination.*

By agglutination of all the strains with one cholera serum — Serum No. 8 — the material was divided in two large groups, the vibrios agglutinable by this serum and those non-agglutinable.

At the same time the strains were agglutinated with an El Tor serum produced from strain 94 — originally marked D_{47} — and with a serum produced from strain 29, which had been received as a non-agglutinable cholera vibrio.

The results of these agglutination tests are shown in Table 13.

Table 13.

Agglutination table.

The strains 1—408 agglutinated with serum 8, serum 29 and serum 94.

The values put down are the reciprocal values of the smallest concentrations of serum which caused macroscopically visible agglutination.

0 indicates that there was not any agglutination with the highest serum concentration examined. This concentration was 16 times that of the last tube showing agglutination with the homologous strain.

According to this the highest concentrations examined were as follows: serum 8: 1/100, serum 29: 1/200 and serum 94: 1/400, the titres of these three sera being: 1600, 3200 and 6400.

Strain	Ser. 8	Ser. 29	Ser. 94	Saline	Strain	Ser. 8	Ser. 29	Ser. 94	Saline
1.	800	0	3200	0	22.	800	0	800	0
2.	1600	0	800	0	23.	800	0	3200	0
3.	1600	0	800	0	24.	0	0	0	0
4.				+	25.	800	0	1600	0
5.				+	26.	0	0	0	0
6.	1600	0	400	0	27.	0	0	0	0
7.	800	0	6400	0	28.	0	0	0	0
8.	1600	0	800	0	29.	0	3200	0	0
9.	1600	0	800	0	30.	0	3200	0	0
10.	800	0	6400	0	31.	1600	0	6400	0
11.	1600	0	3200	0	32.				+
12.	1600	0	1600	0	33.	0	0	0	0
13.	1600	0	1600	0	34.	1600	0	1600	0
14.	1600	0	800	0	35.	0	0	0	0
15.				+	36.	800	0	800	0
16.				+	37.	1600	0	800	0
17.				+	38.	800	0	800	0
18.				+	39.	0	0	0	0
19.	800	0	3200	0	40.	0	0	0	0
20.	800	0	1600	0	41.	800	0	1600	0
21.	0	0	0	0	42.	800	0	800	0

Strain	Ser. 8	Ser. 29	Ser. 94	Saline
43.	1600	0	800	0
44.	1600	0	3200	0
45.	1600	0	800	0
46.	1600	0	800	0
47.	800	0	1600	0
48.	800	0	3200	0
49.	1600	0	3200	0
50.	800	0	1600	0
51.	800	0	800	0
52.	1600	0	800	0
53.	1600	0	1600	0
54.	800	0	800	0
55.	1600	0	800	0
56.	800	0	800	0
57.	1600	0	800	0
58.	800	0	800	0
59.	1600	0	6400	0
60.	0	0	0	0
61.	0	0	0	0
62.	1600	0	3200	0
64.	800	0	800	0
65.	800	0	1600	0
66.	800	0	1600	0
67.	1600	0	3200	0
68.	0	0	0	0
69.	0	(400) (0)	0	0
70.	0	3200	0	0
71.	800	0	1600	0
72.	0	0	0	0
73.	0	0	0	0
74.	1600	0	3200	0
75.	0	0	0	0
76.	0	0	0	0
78.	0	0	0	0
79.	0	0	0	0
80.	800	0	800	0
81.	0	0	0	0
83.	0	0	0	0
84.				+
85.	0	0	0	0
86.	0	0	0	0
87.	1600	0	800	0
88.	0	0	0	0
89.	1600	0	800	0
90.	1600	0	800	0
91.	1600	0	800	0
92.	0	0	0	0
93.	0	0	0	0
94.	800	0	6400	0
95.	1600	0	400	0
96.	800	0	400	0
97.	1600	0	1600	0
98.	800	0	800	0
99.	1600	0	800	0
100.	800	0	3200	0
101.	800	0	3200	0
102.	0	0	0	0
103.				+
104.	0	0	0	0
105.				+
106.	800	0	800	0
107.	800	0	1600	0
108.	800	0	800	0
109.	0	0	0	0
110.	800	0	3200	0
112.	0	0	0	0
113.	800	0	6400	0
114.	800	0	3200	0
115.				+
116.	1600	0	3200	0
117.	400	0	1600	0
118.	800	0	3200	0
119.	800	0	1600	0
120.	800	0	1600	0

Strain	Ser. 8	Ser. 29	Ser. 94	Saline
121.	800	0	1600	0
122.	1600	0	800	0
123.	800	0	800	0
124.	400	0	800	0
125.	800	0	800	0
126.	1600	0	1600	0
127.	800	0	800	0
128.	1600	0	1600	0
129.	1600	0	800	0
130.				+
131.	800	0	1600	0
132.	800	0	1600	0
133.	1600	0	1600	0
134.	800	0	1600	0
135.	800	0	800	0
136.	800	0	1600	0
137.	800	0	1600	0
138.	1600	0	1600	0
139.	800	0	800	0
140.	800	0	1600	0
142.	800	0	1600	0
143.	800	0	1600	0
144.	800	0	1600	0
146.	1600	0	1600	0
147.	800	0	800	0
148.	800	0	800	0
149.	800	0	1600	0
150.	1600	0	1600	0
151.	1600	0	1600	0
152.	800	0	1600	0
153.	800	0	1600	0
154.	1600	0	1600	0
155.	800	0	1600	0
156.	800	0	1600	0
157.	800	0	400	0
158.	800	0	6400	0
159.	0	0	0	0

Strain	Ser. 8	Ser. 29	Ser. 94	Saline
160.	1600	0	1600	0
161.	400	0	6400	0
162.	800	0	800	0
163.	1600	0	1600	0
164.	800	0	800	0
165.	1600	0	800	0
166.	1600	0	1600	0
167.	800	0	800	0
168.	800	0	1600	0
169.	800	0	800	0
170.	1600	0	400	0
171.	400	0	800	0
172.	800	0	800	0
173.	400	0	1600	0
174.	800	0	800	0
175.	800	0	800	0
176.	800	0	1600	0
177.	1600	0	800	0
178.	800	0	800	0
179.	800	0	3200	0
180.	800	0	1600	0
181.	800	0	1600	0
183.	800	0	3200	0
184.	1600	0	1600	0
185.	800	0	3200	0
186.	800	0	400	0
187.	800	0	800	0
188.	1600	0	800	0
189.	1600	0	800	0
190.	800	0	1600	0
191.	1600	0	200	0
192.	800	0	800	0
193.	1600	0	800	0
194.	1600	0	3200	0
195.	1600	0	800	0
196.	800	0	1600	0
197.	1600	0	1600	0

Strain	Ser. 8	Ser. 29	Ser. 94	Saline
198.	800	0	800	0
199.	1600	0	800	0
200.	1600	0	800	0
201.	800	0	3200	0
202.	1600	0	1600	0
203.	800	0	3200	0
204.	0	0	0	0
206.	1600	0	1600	0
207.	1600	0	800	0
208.	1600	0	800	0
209.	800	0	3200	0
210.	1600	0	800	0
211.	1600	0	1600	0
212.	1600	0	800	0
213.	800	0	1600	0
214.	1600	0	1600	0
215.	1600	0	6400	0
216.	1600	0	3200	0
217.	1600	0	1600	0
218.				+
219.	1600	0	6400	0
220.	400	0	1600	0
221.	400	0	3200	0
222.	800	0	800	0
223.	800	0	6400	0
224.	800	0	400	0
225.	800	0	3200	0
226.	800	0	3200	0
227.	800	0	(400)	0
228.	800	0	1600	0
229.	800	0	800	0
230.	1600	0	400	0
231.	800	0	800	0
232.	800	0	800	0
233.	800	0	800	0
234.	1600	0	200	0
235.	1600	0	1600	0

Strain	Ser. 8	Ser. 29	Ser. 94	Saline
236.	1600	0	1600	0
237.	1600	0	800	0
238.	1600	0	1600	0
239.				+
240.	800	0	6400	0
241.	800	0	3200	0
242.	1600	0	1600	0
243.	800	0	800	0
245.	1600	0	800	0
246.	1600	0	800	0
247.	1600	0	1600	0
248.	800	0	3200	0
249.	400	0	3200	0
250.	800	0	800	0
251.	0	0	0	0
252.	(100)	0	0	0
253.	800	0	1600	0
254.	400	0	0	0
256.	400	0	1600	0
257.	1600	0	1600	0
258.	800	0	1600	0
259.	0	0	0	0
260.				+
261.	1600	0	1600	0
262.	1600	0	1600	0
263.	800	0	800	0
264.	1600	0	1600	0
265.	800	0	1600	0
266.	800	0	800	0
267.	0	1600	0	0
268.				+
269.	800	0	1600	0
271.	800	0	3200	0
275.	800	0	1600	0
276.	800	0	1600	0
277.	400	0	1600	0
279.	800	0	1600	0

Strain	Ser. 8	Ser. 29	Ser. 94	Saline
280.	800	0	3200	0
281.	400	0	1600	0
282.	0	0	0	0
283.	0	0	0	0
284.	0	1600	0	0
285.	0	0	0	0
286.	0	0	0	0
287.	400	0	1600	0
288.	0	0	0	0
289.	1600	0	1600	0
290.	1600	0	3200	0
291.	0	0	0	0
292.	0	0	0	0
293.	0	0	0	0
294.	0	0	0	0
295.	0	0	0	0
296.	0	0	0	0
297.	0	0	0	0
298.	0	0	0	0
299.	0	0	0	0
300.	0	0	0	0
301.	0	0	0	0
302.	0	0	0	0
303.	0	0	0	0
304.	0	400	0	0
306.	0	0	0	0
307.	0	0	0	0
308.	0	0	0	0
309.	0	0	0	0
310.	0	1600	0	0
311.	0	200	0	0
312.	0	0	0	0
313.	0	0	0	0
314.	0	0	0	0
315.	0	0	0	0
316.	0	0	0	0
317.	0	0	0	0
318.	0	0	0	0
319.	0	0	0	0
320.	0	0	0	0
321.	100	0	0	0
322.				+
323.	0	0	0	0
324.	0	0	0	0
325.	0	0	0	0
326.	0	0	0	0
327.	0	0	0	0
328.	0	—	0	0
329.	0	0	0	0
330.	0	800	0	0
331.	0	800	0	0
332.	0	0	0	0
333.	0	0	0	0
334.	0	0	0	0
335.	0	0	0	0
336.	0	0	0	0
337.	0	0	0	0
338.	0	800	0	0
339.	0	0	0	0
340.	0	0	0	0
341.	0	0	0	0
342.				(+)
343.	0	0	0	0
344.	0	0	0	0
345.	0	0	0	0
346.	0	0	0	0
347.	0	0	0	0
348.	0	0	0	0
349.	0	0	0	0
350.	0	0	0	0
351.	0	0	0	0
352.	0	0	0	0
353.	0	800	0	0
354.	0	0	0	0

Strain	Ser. 8	Ser. 29	Ser. 94	Saline	Strain	Ser. 8	Ser. 29	Ser. 94	Saline
355.	0	0	0	0	383.	0	200	0	0
356.	0	0	0	0	384.	0	0	0	0
357.	0	0	0	0	385.	800	0	400	0
359.	800	0	800	0	386.	0	0	0	0
360.	800	0	6400	0	387.	0	0	0	0
361.				+	388.	0	0	0	0
363.	800	0	6400	0	389.	0	0	0	0
364.	800	0	1600	0	390.	800	0	3200	0
365.	800	0	3200	0	391.	800	0	1600	0
366.	1600	0	800	0	392.	800	0	1600	0
368.	1600	0	6400	0	393.	0	0	0	0
369.	400	0	1600	0	394.	0	0	0	0
370.	1600	0	800	0	396.	0	0	0	0
371.	1600	0	3200	0	397.	1600	0	800	0
372.	800	0	1600	0	398.	1600	0	800	0
373.	1600	0	800	0	399.	1600	0	3200	0
374.	1600	0	800	0	400.	800	0	1600	0
375.	1600	0	800	0	401.	800	0	3200	0
376.	800	0	1600	0	402.	800	0	1600	0
377				+	403.	800	0	800	0
378.	800	0	1600	0	404.	800	0	3200	0
379.	800	0	800	0	405.	1600	0	3200	0
380.	0	400	0	0	407.	0	0	0	0
381.	0	0	0	0	408.	0	0	0	0
382.	0	0	0	0					

As soon as these tests had been carried out the following questions arose: Is there any relation between the types determined on the basis of the fermentation reactions and the agglutinability of the strains towards a certain cholera serum, in this case Serum 8? Are the agglutinable and non-agglutinable strains distributed at random in all groups or do the definitions of the fermentative types correspond to the serological grouping? If the last were the case, the importance of the fermentative reaction would be greatly increased. A table was therefore constructed in which the strains within each of the fermentative types were divided in 3 groups, those agglutinable with cholera serum, those non-agglutinable, and those

spontaneously agglutinable. Table 14 gives the results of this grouping*).

Table 14.

	Type					
Serum 8	I	II	III	IV	V	VI
agglutinable	245	1	0	0	0	0
non-agglutinable	25	73	11	3	2	5
spontaneously agglutinable..	17	1	1	0	0	0

Grouping of the strains according to their fermentative type and relation to Serum 8 (cholera serum).

The table shows that with one single exception — Strain 254, V. Dunbar — all the agglutinable vibrios fermented as type I. This behaviour is of no small importance, as it shows that we may *exclude* with almost certainty the possibility that with the technique used here vibrios giving a fermentation reaction of types II—VI are agglutinable with cholera serum.

As already mentioned Vibrio Dunbar is an exception to this rule. The Vibrio Dunbar seems to have been isolated by Dunbar at Hamburg about 40 years ago from the river water of the Elbe at a period when cholera was not known to appear in the neighbourhood of the Elbe. Its agglutination reactions with cholera serum vary somewhat, the agglutination with the same serum sometimes taking place in dilutions T/16, sometimes only in T/32 or T/64; the two latter dilutions are outside the limits of dilutions generally used by me whilst the dilution T/16 is the one used in the first tube. This shows that the vibrio in question only agglutinates poorly with cholera serum; it also happened that serum made from V. Dunbar only agglutinated cholera vibrios poorly.

A cross absorption experiment — technique p. 107, experimental routine p. 154, — between the strains 8 and 254 and the corresponding sera showed, as seen in table 15, that none of these strains remove all the agglutinin from the serum of the other, and

*) A few of the groups in this table differ in number from previous results published by the author (1); this is partly due to the fact that more strains have been used in the present investigation partly to a few strains being lost during the final part of this work. A comparison shows that in the main the differences are of no importance.

that they are only able to do so from their own serum. This probably indicates that the two strains possess a mutual antigen which is not very strongly agglutinogenic.

Table 15.

Serum	Antigen	dilutions of serum 100	200	400	800	1600	Saline
8/0	8	3	3	3	2	1	0
8/0	254	3	3	2	0	0	0
8/8	8	0	0	0	0	0	0
8/254	254	0	0	0	0	0	
8/254	8	3	3	3	2	1	

		dilutions of serum 400	800	1600	3200	6400
254/0	254	3	3	3	1	0
254/0	8	2	1	0	0	0
254/254	254	0	0	0	0	0
254/8	8	0	0	0	0	0
254/8	254	3	3	3	0	0

Cross absorption test between strain 8 — a V. Choleræ — and strain 254 — V. Dunbar.

For explanation of signatures see p. 154.

These various findings make it likely that the V. Dunbar is a cholera-like vibrio.

It is an exception to the rule that a vibrio agglutinable with cholera serum can ferment mannose. It may at any rate be said that it constitutes a "lucky" exception, as it is probably not a true cholera vibrio; at any rate it has never been regarded as such.

Table 14 moreover shows that not every vibrio which ferments according to type I is agglutinable with cholera serum.

The fermentative type of the vibrios agglutinable with cholera serum found in these experiments, agree, with one single exception, with the fermentative reactions of these strains as described in the literature. As previously mentioned, Nobechi found a single strain

which did not ferment saccharose but was agglutinable with cholera serum; as in this case it was an old laboratory strain which also showed abnormality in lacking motility I believe that it is justifiable to consider this an exception which does not diminish the importance of the fermentative types in practical investigations. Nobechi's material also contained 2 strains, which according to my classification ferment like type II, and one strain that fermented like type III. According to Nobechi these three strains were non-agglutinable with cholera serum.

From table 13 it is seen that Serum 8 and Serum 94 agglutinate practically the same strains. Only two strains, Nos. 191 and 234 were not agglutinable by Serum 94 in the dilutions used here. By examination in lesser dilution it was found that the titre of Serum 94 against these two strains was 200.

The agreement between the two Sera 8 and 94 only appears as far as agglutination or non-agglutination is concerned. In regard to the dilution of serum which agglutinates the different strains, Serum 8 causes agglutination up to the last tube but one with nearly all the strains, whilst the agglutination of Serum 94 varies greatly, from agglutination in all 5 tubes to agglutination in the first tube only. This seems to indicate that the two sera are of different quality, and not identical. This will be discussed in more detail on p. 156. Here it is enough to emphasize that no strain is agglutinated by Serum 94 without also being agglutinated by Serum 8.

As regards Serum 29 it will be seen that 14 strains in all are agglutinated by this serum, and that these strains are non-agglutinable with the Sera 8 and 94.

Amongst the strains non-agglutinable with Serum 8 some were chosen at random for the production of serum. These sera were tried with agglutination tests against the strains that were non-agglutinable with Serum 8. This made it possible to delimit new groups of vibrios which were agglutinable with some of these sera. Again, sera were produced from the strains that so far had not been agglutinated, and these sera were tested against the non-agglutinated strains. The experiments were carried on in this manner until nearly all the strains had been agglutinated by one or more serums.

If these groups, defined by their agglutinability, are placed in the fermentation table, groups to be seen in table 16 are formed.

Table 16.

Table 16.

Table showing the strains grouped according to fermentation reactions and agglutinability.

	I + Mn, + Sa, 0 Ar	II 0 Mn, + Sa, 0 Ar	III + Mn, + Sa, + Ar	IV 0 Mn, + Sa, + Ar	V + Mn, 0 Sa, 0 Ar	VI 0 Mn, 0 Sa, 0 Ar
Serum 8	*See Table 16 a*	(254)				
— 292	81 102 292 309 334					
— 340	291 340					
— 301	301					
— 286	286					
— 33	33	75 104				
— 282	282 314 325 384	39 302 346 347 356 381 382 386 408				
— 29	70	29 30 284 267 304 310 311 330 331 338 353 380 383				
324	294 313 316 317 333	21 93 324				
		73 159 288 299 300				

— 68		68 296 350				
— 204		204 332 389				
— 298		60 298				
— 341		307 341				
— 388		388				
— 27		27				
— 26		26				
— 303			88 303 306 351			
— 24			295			24
— 35			35			
— 72				72		
— 40					40	
Spontaneous agglutination	4 5 15 16 17 18 32 103 105 115 130 218 239 260 268 377 361	335	342			
Non-grouped	61 69 76 82 252	28 84 85 86 109 285 323 327 328 337 348 357 407	283 293 318 321 336	305 349	354	78 83 251 297

Table 16 a.

The group of strains which in Table 16 belong to type I, serum 8.

	I + Mn, + Sa, 0 Ar
Serum 8.	1 2 3 6 7 8 9 10 11 12 13 14 19 20 22 23 25 31 34 36 37 38 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 62 64 65 66 67 71 74 80 87 89 90 91 94 95 96 97 98 99 100 101 106 107 108 110 113 114 116 117 118 119 120 121 122 123 124 125 126 127 128 129 131 132 133 134 135 136 137 138 139 140 142 143 144 146 147 148 149 150 151 152 153 154 155 156 157 158 160 161 162 163 164 165 166 167 168 169 170 171 172 173 174 175 176 177 178 179 180 181 183 184 185 186 187 188 189 190 191 192 193 194 195 196 197 198 199 200 201 202 203 206 207 208 209 210 211 212 213 214 215 216 217 219 220 221 222 223 224 225 226 227 228 229 230 231 232 233 234 235 236 237 238 240 241 242 243 244 245 246 247 248 249 250 253 256 257 258 261 262 263 264 265 266 269 271 275 276 277 279 280 281 287 289 290 322 359 360 363 364 365 366 368 369 370 371 372 373 374 375 376 378 379 385 390 391 392 397 398 399 400 401 402 403 404 405

The first vertical column indicates the sera used for agglutination and the following the different types of fermentation.

Like other investigators, the author found that the vibrios may be divided in regard to their serological reactions into many groups. Each of the groups in the table will now be discussed.

Numerically the material examined here may be divided into one large group of vibrios, agglutinated by serum produced from a cholera vibrio, and numerous smaller groups, each including from 15 to one single strain. These figures, based on a material gathered

entirely at random, gives no information whatever about the numerical relation between the different groups as found in nature. Nevertheless, it is not entirely without interest to examine the strains included in each separate group.

The largest group — including the vibrios which ferment like type I and are agglutinated by Serum 8 — contains amongst other strains all the classic cholera vibrios of the material, e. g. V. Choleræ Pfeifferi, V. Choleræ Pottevin, V. Choleræ Jenkins, Kabeshimas type "original" and many others. Moreover it includes strains isolated from clinical cases of cholera in British India, Siam, Java, Indochina, Southern and Northern China, Manila, Japan and Africa, and also a few strains isolated in Europe.

This shows that in all countries in which cholera is or has been an important epidemiological factor it has been possible to isolate vibrios agglutinable with a serum produced from what is considered to be a typical strain of true cholera, in this case a vibrio isolated from a clinical case of cholera confirmed by autopsy. This strain is itself agglutinated by the classical cholera sera. Cross absorption experiments with some of the other strains, which according to their origin should be typical cholera vibrios, and their sera, showed in three cases that the antigenic factors were identical with the factors of strain 8 (see Table 17).

From this we cannot deduce, however, that all the strains of this group contain a common factor. If Serum 8 contains different kinds of agglutinin, corresponding to the factors of strain 8, it is quite possible that some of the strains of the group were agglutinated by one factor, other strains by another factor.

Moreover this group contains the Japanese strain type intermédiaire in addition to the type original already mentioned.

Type varié also belongs to the fermentative type I, but it is agglutinable with S. 340 (see Table 16). Cross agglutination and cross absorption experiments with my usual technique have not given me cause to regard, like Nobechi for instance, these 3 strains as three different, but related, types.

As seen in Table 18, type original and type intermédiaire were able to remove all the agglutinin from each other's sera on cross ab-

Table 17.

A. Strain 8 and strain 289.

Serum	Antigen	Dilutions of serum 100	200	400	800	1600	Saline.
8/0	8	3	3	3	1	0	0
8/0	289	3	3	3	2	0	0
8/8	8	0	0	0	0	0	
8/289	289	0	0	0	0	0	
8/289	8	0	0	0	0	0	
		50	100	200	400	800	
289/0	289	3	3	3	3	2	
289/0	8	3	3	3	3	2	
289/289	289	0	0	0	0	0	
289/8	8	0	0	0	0	0	
289/8	289	0	0	0	0	0	

B. Strain 8 and strain 368.

		100	200	400	800	1600	
8/0	8	3	3	3	3	1	0
8/0	368	3	3	3	0	0	0
8/8	8	1	0	0	0	0	
8/368	368	0	0	0	0	0	
8/368	8	(1)	(1)	(1)	0	0	
		200	400	800	1600	3200	
368/0	368	3	3	3	2	1	
368/0	8	3	3	3	2	1	
368/368	368	0	0	0	0	0	
368/8	8	0	0	0	0	0	
368/8	368	0	0	0	0	0	

(Cont. next page.)

C. Strain 8 and strain 369.

		Dilutions of serum					
		100	200	400	800	1600	
8/0	8	3	3	3	3	2	0
8/0	369	3	3	3	3	2	0
8/8	8	0	0	0	0	0	
8/369	369	0	0	0	0	0	
8/369	8	0	0	0	0	0	

		200	400	800	1600	3200	6400
369/0	369	3	3	3	3	2	1
369/0	8	3	3	3	2	1	0
369/369	369	0	0	0	0	0	0
369/8	8	0	0	0	0	0	0
369/8	369	0	0	0	0	0	0

Cross absorption experiments between strain 8 and the strains 289, 368 and 369.

For explanation of the signatures see p. 154.

sorption; and apparently type varié did not contain any of the factors either of type original or of intermédiaire.

I cannot explain all the lack of accord between the Japanese investigations and mine. The different Japanese investigations, however, also show somewhat divergent results. In Kabeshima's reports on type original and type varié it was stated that the agglutinability of these strains varied greatly towards the serum of one another. Nobechi, on the other hand, found that serum original and serum intermédiaire agglutinated all three strains in the same dilution, whilst serum varié agglutinated intermédiaire a little and original a goods deal less than the homologous strain.

Kuroya and Ōno, who worked with the same strains as Nobechi, found with the sera they produced, that serum original agglutinated strain intermédiaire up to 1/4 and strain varié only to 1/20

Table 18.

A. *Serum 8 and the strains 289 (T. original), 290 (T. intermédiaire), 291 (T. varié).*

	Serum 8, Serum dilutions					
	100	200	400	800	1600	Saline
Strain No. 289	3	3	3	2	1	0
— - 290	3	3	3	2	1	0
— - 291	0	0	0	0	0	0

B. *Cross absorption between strain 8 and strain 289.*

		Serum dilutions					
Serum	Antigen	100	200	400	800	1600	Saline
8/0	8	3	3	3	2	0	0
8/0	289	3	3	3	2	0	0
8/8	8	0	0	0	0	0	
8/289	289	0	0	0	0	0	
8/289	8	0	0	0	0	0	
		50	100	200	400	800	
289/0	289	3	3	3	3	2	
289/0	8	3	3	3	3	2	
289/289	289	0	0	0	0	0	
289/8	8	0	0	0	0	0	
289/8	289	0	0	0	0	0	

C. *Cross absorption between strain 289 and strain 290.*

		Serum dilutions				
Serum	Antigen	100	200	400	800	1600
289/0	289	3	3	3	2	0
289/0	290	3	3	3	2	0
289/289	289	0	0	0	0	0
289/290	290	0	0	0	0	0
289/290	289	0	0	0	0	0
290/0	290	3	3	3	2	0
290/0	289	3	3	3	1	0
290/290	290	0	0	0	0	0
290/289	289	0	0	0	0	0
290/289	290	0	0	0	0	0

Explanation of signatures see p. 154.

of the titre. Serum intermédiaire agglutinated strain original to 1/4 and varié to 1/10 of the titre, whilst serum varié agglutinated strain original up to 1/20 and intermédiaire to 1/4 af the titre. Kuroya and Ôno did not use a magnifying glass for their readings; the bacterial concentration in their tubes was, as far as can be ascertained, considerably below the concentration used by me.

Whilst the results of my agglutination reactions do not agree with those of Nobechi, the disagreement between my results and those of Kuroya and Ono apparently is far less, as I did not examine serum dilutions below 1/16. This at any rate explains that, in apparent contrast to Kuroya and Ono, I found the strains original and varié not agglutinated by the serum of one another; this added to the fact that I used a greater concentration of bacteria explains why the strain varié was found to be non-agglutinable with serum intermédiaire.

The divergence in the results of the agglutination of the strain intermédiaire with the serum varié cannot be explained categorically.

This group, in addition to the vibrios that have been isolated from clinical cases of undoubted cholera, also contains some vibrios, namely the El Tor strains, which, as previously discussed, have been isolated in circumstances that make it justifiable to doubt whether they really are pathogenetic cholera vibrios.

Several of these strains were examined by absorption with the view of finding special antigenic factors which were not represented amongst the rest of the vibrios agglutinable with cholera serum. The results of these experiments may be seen on p. 156. Amongst other things it was found that strain 94 contained one antigen group more than strain 8. If this fact be compared with the agglutination results of Table 13 which showed that all the strains agglutinated by Serum 94, are also agglutinated by Serum 8, it will be seen, that the special factor of strain 94 is not found in any strain which does not also contain some of the antigen present in strain 8. The absorption experiments showed further that the special factor found in strain 94 was also present in several other strains in addition to the El Tor strains and in a few of the strains isolated from cholera patients. This will be further discussed on p. 164.

On the whole it may be said that this group includes specimens

which definitely belong to the species of bacteria which have been called V. Choleræ since Koch's discovery in 1893. It includes at least two sub-groups which may be distinguished serologically.

On the basis of the experiments carried out here it must be stated that at present it is impossible, at any rate with the technique used in these experiments, to distinguish in the laboratory between the strains isolated at El Tor and other strains, which without doubt are true cholera vibrios.

The only vibrio which fermented like a type other than type I and yet was agglutinated by cholera serum, is the *Vibrio Dunbar* which ferments like type II. The behaviour of this vibrio has been discussed in greater detail on p. 114.

The remaining groups of Table 16 are all relatively small. They may be divided into those which include vibrios of only one fermentative type, and those which include vibrios of varying fermentative types.

In regard to their relationship to the V. Choleræ the strains that ferment like type I are of the greatest interest. There are 4 serological »groups« which contain representatives of this fermentative type alone, i. e. the 5 strains which were agglutinated by serum 292, 2 strains agglutinated by serum 340 and the strains 301 and 86, which were agglutinated only by their own serum. To these may be added the non-agglutinated strains and the spontaneously agglutinable strains.

The five strains which were agglutinated by Serum 292 were: 81 and 102, both isolated at El Tor in 1930 and 1931 with the registration marks D_{64} and D_{20}, and also 292, 309 and 334, all three isolated at Calcutta from cholera-like cases on Jan. 23rd, Aug. 24th and May 1st, 1932.

Serum 340, besides agglutinating strain 340, which was isolated from a cholera-like case in Calcutta on Feb. 11th, 1931, also agglutinates strain 291. The latter strain is identical with the type varié of Kabeshima and Nobechi. As already mentioned, this is the type isolated during the epidemics of clinical cholera at Shanghai in 1932. According to the investigations reported, this vibrio must be able to produce a disease, which clinically can hardly be distinguished from cholera. It seems quite strange that amongst the strains received

from Calcutta there were not more belonging to this group. But the strain 340 proves that in British India, as in China and Japan, where they have previously been found, there may also be found strains which have a common antigen to type varié.

The strain 301, which is only agglutinated by Serum 301, has been received from Calcutta, where it was isolated on May 4th, 1932.

The strain 286, which is also agglutinated by its own serum alone, was isolated at Manila in 1925 as a »non-agglutinable« cholera vibrio from a cholera-like case.

The remaining 4 serological groups, which include strains of the fermentative type I, also include strains which ferment like type II.

Serum 33 agglutinates its homologous strain, which was sent to us as a cholera vibrio, non-agglutinable with Serum 8, but agglutinable with the serum used where it was isolated, at Kasauli in Northern India. Information as to its origin is lacking; it ferments like type I; another 2 strains belonging to type II are agglutinated by Serum 33: the strain 75, which was isolated on Aug. 5th, 1929 in Calcutta, and the strain 104, isolated in 1930—1931 at El Tor, bearing the symbol D_{49}. Cross absorption experiments have shown the strains 33 and 75 to be identical, see table 19, a fact which is of some interest, as it shows that differences in fermentative type do not necessarily indicate differences in antigenic composition; an analogous relationship is known in the salmonella group.

Serum 29 agglutinates one strain of type I, the vibrio paracholeræ A, Mackie and Storer; cross absorption tests between the latter strain No. 70 and strain 29 have shown that they are probably identical. Table 20 gives the results of a cross absorption experiment between these two strains and their sera.

Table 19 and Table 20, see next page.

Table 19.

		Serum dilutions					
Serum	Antigen	200	400	800	1600	3200	Saline
33/0	33	3	3	3	3	1	0
33/0	75	3	3	2	1	0	0
33/33	33	0	0	0	0	0	
33/75	75	0	0	0	0	0	
33/75	33	0	0	0	0	0	
Serum	Antigen	400	800	1600	3200	6400	
75/0	75	3	3	2	1	0	
75/0	33	3	3	3	1	0	
75/75	75	0	0	0	0	0	
75/33	33	0	0	0	0	0	
75/33	75	0	0	0	0	0	

Cross absorption test between strain 33 and strain No. 75.
For explanation of signatures see p. 154.

Table 20.

		Serum dilutions						
Serum	Antigen	100	200	400	800	1600	3200	Saline
29/0	29	3	3	3	2	1	1	0
29/0	70	3	3	2	2	1	0	0
29/29	29	0	0	0	0	0	0	
29/70	70	0	0	0	0	0	0	
29/70	29	0	0	0	0	0	0	
70/0	70	3	3	3	3	1	0	
70/0	29	3	3	3	1	0	0	
70/70	70	0	0	0	0	0	0	
70/29	29	0	0	0	0	0	0	
70/29	70	2	1	0	0	0	0	

Cross absorption between strain No. 29 and strain No. 70.
For explanation of signatures see p. 154.

This experiment shows that strain 70 removes all the agglutinin from serum 29, which indicates that all the antigens of strain 29 are present in strain 70. Serum 70 is nearly, but not totally exhausted by strain 29, which means that strain 70 contains an antigen more than strain 29 — but the agglutinogenous power of this antigen is so weak, that I cannot definitely say that the above results indicate a true difference between these two strains.

13 other strains within type II are agglutinated by Serum 29 — 29 and 30 were both isolated from the same cholera patient at Manila in 1930, on the first and second days of the disease — strain 284 is possibly also identical in origin with one of these strains, but it has been sent to us from another institute —; 267 has only been marked El Tor D; 304, 310, 311, 330, 331, 338 and 353, isolated at Calcutta from cholera-like cases, on Apr. 30th, June 28th, May 5th, May 1st, and June 28th, 1932 and Feb. 9th, Sept. 16th, 1931; 380 and 383 marked Nanking 56/1932 and 123/1932. All these vibrios were sent to us marked "non-agglutiable vibrios". Cross absorption between 29 and 331 (see table 21) and between 29 and 304 (see table 22) show that these strains are identical.

Table 21.

		Serum dilutions						
Serum	Antigen	100	200	400	800	1600	3200	Saline
29/0	29	3	3	3	3	3	2	0
29/0	331	3	2	1	0	0	0	0
29/29	29	0	0	0	0	0	0	
29/331	331	0	0	0	0	0	0	
29/331	29	0	0	0	0	0	0	
331/0	331	3	3	3	2	1	0	
331/0	29	3	3	3	3	3	2	
331/331	331	0	0	0	0	0	0	
331/29	29	0	0	0	0	0	0	
331/29	331	0	0	0	0	0	0	

Cross absorption test between strain No. 29 and strain No. 331.
Explanation of signatures see p. 154.

Table 22.

Serum	Antigen	Serum dilutions 200	400	800	1600	3200	saline
29/0	29	3	3	3	3	2	0
29/0	304	3	3	2	1	0	0
29/29	29	0	0	0	0	0	
29/304	304	0	0	0	0	0	
29/304	29	0	0	0	0	0	
		50	100	200	400	800	
304/0	304	3	3	3	3	1	
304/0	29	3	3	3	3	2	
304/304	304	0	0	0	0	0	
304/29	29	0	0	0	0	0	
304/29	304	0	0	0	0	0	

Cross absorption test between strain No. 29 and strain No. 304.
Explanation of signatures see p. 154.

Serum 282 agglutinates 4 strains of type I and 9 strains of type II; of type I No. 282, isolated at Manila from a cholera-like case in 1925 as a "non-agglutinable vibrio", 314 and 325 both isolated on Aug. 25th, 1932 at Calcutta from cholera-like cases, and 384, marked Nanking 1932, No. 124; further information is lacking; of type II: 39, received from Kasauli as being non-agglutinable with Serum 8, but agglutinable with Kasauli serum, 302, 346, 347 and 356 isolated at Calcutta from cholera-like cases on Aug. 31st, 1931, Sept. 17th, 1929, May 15th, 1930 and June 23rd, 1931 and finally 381, 382, 386 isolated at Nanking 1932 and 408 most probably isolated in China in 1932. This group thus represents geographically different places.

Table 23.

Serum	Antigen	Serum dilutions 400	800	1600	3200	6400	saline
39/0	39	3	3	3	3	1	0
39/0	282	3	3	3	3	3	0
39/39	39	0	0	0	0	0	
39/282	282	0	0	0	0	0	
39/282	39	0	0	0	0	0	
		50	100	200	400	800	
282/0	282	3	3	3	3	3	
282/0	39	3	3	3	3	2	
282/282	282	0	0	0	0	0	
282/39	39	0	0	0	0	0	
282/39	282	0	0	0	0	0	

Cross absorption test between strain No. 39 and No. 282.
Explanation of signatures see p. 154.

Cross absorption showed that the strains 39 and 282 were identical, (see Table 23). Absorption of Serum 39 with the strains 314, 346, 381 and 408 (see Table 24) showed that strain 314 and strain 346 contained all the antigens of strain 39, whilst strain 381 and strain 408 contained part of the same antigens. Strain 314 and 346 can absorb serum 39 totally in contrast to strain 381 and strain 408, which can remove part of the agglutinins only.

Table 24.

Serum	Antigen	Serum dilutions 200	400	800	1600	3200	6400	saline
39/0	39	3	3	3	3	3	2	0
39/0	314	3	3	3	3	3	3	0
39/314	314	0	0	0	0	0	0	
39/314	39	0	0	0	0	0	0	

Serum	Antigen	Serum dilutions 200	400	800	1600	3200	6400	saline
39/0	346	3	3	3	3	3	3	0
39/346	346	2	0	0	0	0	0	
39/346	39	2	0	0	0	0	0	
39/0	381	3	3	3	3	2	0	0
39/381	381	0	0	0	0	0	0	
39/381	39	3	3	3	3	2	0	
39/0	408	3	3	3	3	0	0	0
39/408	408	0	0	0	0	0	0	
39/408	39	3	3	3	3	3		

Absorption experiment between serum 39 and the strains Nos 314, 346, 381 and 408.

Explanation of signatures see p. 154.

Serum 324 agglutinated 5 strains of type I and 3 of type II. The former were 294, 313, 316, 317 and 333 isolated from cholera-like cases at Calcutta on Aug. 25th, June 28th, June 28th, June 28th and May 28th, 1932; the 3 strains of type II are as follows: 21, isolated at Manila from a cholera-like case in 1930, 93 isolated from water at Calcutta 1931 and 324 isolated at Calcutta from fæces on May 5th, 1932. The strains 313, 316 and 317 were thus isolated on the same day at Calcutta. Absorption of Serum 324 with 313 and 333 (see Table 25) shows that all the antigens of strain 324 are present in strain 313 and strain 333.

Table 25.

Serum	Antigen	Serum dilutions 200	400	800	1600	3200	saline
324/0	324	3	3	3	3	2	0
324/0	313	3	3	3	3	2	0
324/313	313	0	0	0	0	0	
324/313	324	0	0	0	0	0	

Serum	Antigen	Serum dilutions 200	400	800	1600	3200	saline
324/0	333	3	3	3	3	2	0
324/333	333	0	0	0	0	0	
324/333	324	0	0	0	0	0	

Experiment of absorption of the serum 324 with the strains Nos 313 and 333.

Explanation of signatures see p. 154.

As previously stated, the strains that were agglutinable with Serum 8 were not examined as to their agglutinability with sera produced from strains that were non-agglutinable with Serum 8. It was therefore still theoretically possible, that some of the strains, besides containing the antigen group of Serum 8, also contained one or several other antigen groups, possibly represented amongst the strains that were non-agglutinable with Serum 8. The following example illustrates this statement: If the antigen of strain 8 is called A, the antigen of another strain agglutinable with Serum 8 is called AX, and the antigen of a strain non-agglutinable with Serum 8 X, it will be seen that a serum produced from X will agglutinate AX, but not A. The experiments carried out so far do not exclude the possibility of similar relationships in the material examined.

In order to examine whether the strains agglutinable with Serum 8 contained antigenic factors also found outside this group, all the strains had agglutination tests carried out on a microscopic slide with the sera which otherwise agglutinated strains of type I, as it appeared most likely that possible common factors would be found amongst the strains that had been used for the production of sera of this type. There were indeed, as already mentioned, some sera which agglutinated strains of both type I and II, but in the cases in which this had been tested, serological identity was proved between the strains belonging to these two types. The following sera were used: S 292, S 340, S 301, S 33, S 286, S 282, S 70 (identical with S 29), S 68 and S 34, the latter representing an ordinary cholera serum.

This investigation showed that all the strains were agglutinable with Serum 34 and non-agglutinable with S 292, S 340, S 301, S 33, S 282, S 70 and S 68. Only one serum, Serum 286, showed re-

lationships that might indicate the presence of a factor common to strain No 286 and a few of the strains examined, 32 strains being agglutinated more or less by this serum. This possibly indicates that No. 286 has an antigen common to true cholera vibrios. As the titre of this common agglutinin upon titration and agglutination with the 32 strains mentioned was fairly low, I cannot speak dogmatically on the importance of this finding. Perhaps it is a non-specific antigen, which has appeared during laboratory cultivation.

Thus with the exception of No. 286, common factors have not been found in any of the 7 serologically different strains of the fermentative type I and 246 strains which serologically and biochemically belong to the group of vibrios called V. Choleræ.

5 strains, fermenting like type I were not agglutinated by the sera used. They are strain 61, called V. Metchnikow-Bordet, but othervise of unknown origin, strain 69, called V. paracholeræ Martin, strain 82, called V. cholera-like, received from N. C. T. C., strain 252, isolated at El Tor 1930—1931, marked D_{16} and lastly a strain that was sent to us with the name of cholera vibrio, strain 76. Unfortunately serum has not been made from this strain and its reactions have not been examined. It is theoretically possible that this strain was previously agglutinable, and has now become non-agglutinable; on the other hand, information as to earlier examinations is lacking.

We have now discussed the groups including the strains belonging to type I and some of those belonging to type II. Moreover type II includes one group of 15 strains, one group of 5, 3 groups of 3 strains, 2 groups of 2 strains and 3 "groups" with only a single strain; moreover, there are 13 strains which were not agglutinated by any of the sera used here; but nothing indicates that these strains are definitely non-agglutinable. 1 strain is spontaneously agglutinable.

12 out of the 15 strains agglutinated by Serum 345 have been isolated at Calcutta from cholera-like diseases. Their numbers are: 73, 159, 299, 300, 308, 312, 320, 329, 339, 344, 345 and 355, isolated in 1929 and on Jan. 24th, Feb. 20th, Jan. 24th, Feb. 15th, Jan. 16th, March 8th and Feb. 6th, 1933, July 18th and July 23rd, 1929, Feb. 6th, 1933 and May 5th, 1930. The strains N^{os} 393 and 394 have been sent to us from Shanghai, No. 288 has been isolated at Manila, further information is lacking.

One of the 5 strains agglutinated by Serum 112 is strain No. 259, bearing the marking V. Metchnikow; but further information as to its origin is not available; neither this strain, nor the other bearing the same mark — strain No. 60 — showed pathogenicity on intramuscular injection in doves. 3 other strains in this group are from Calcutta, these being strains N^{os} 79, 112 and 352, isolated in 1929, on March 8th, 1929 and on June 23rd 1931. The fifth strain, strain No. 387, was sent from Nanking.

In addition to its homologous strain, Serum 68, produced from V. paracholeræ B (Mackie and Storer), agglutinates the strains No. 296 and 350, both isolated from cholera-like cases at Calcutta, on April 18th. and on May 22nd, 1932. Cross absorption tests showed that No. 296 and No. 68 are identical, and by absorption Serum 68 was found to be exhausted by strain No. 350. This shows a group of vibrios, isolated from patients with diarrhoea at places so far apart as Egypt and Calcutta, in which 2 strains at any rate were found identical.

The strains N^{os} 204, 332 and 389 were agglutinated by Serum 204. The two strains first mentioned were sent at different times with the same mark, No. 1695, isolated on March 10th, 1933, from the same laboratory, i. e. they are identical as to their origin, being isolated from the fæces of a patient with a cholera-like disease at Calcutta. Strain No. 389 was sent to us from Shanghai as a water vibrio 110/1932.

Serum 298 agglutinated the strains N^{os} 298 and 60; the former had been isolated at Calcutta on Sept. 7th, 1932 from a cholera-like disease, the latter being an old laboratory strain belonging to the museum of the Serum Institute, bearing the mark V. Metchnikow.

N^{os} 307 and 341, which form a separate group, were both isolated from cholera-like cases at Calcutta, on July 30th, 1932 and on Sept. 25th, 1929.

There are 3 single strains non-agglutinable except with their own sera. They are N^{os} 388, 27 and 26; 27 and 26 were isolated at Manila in 1924, whilst No. 388 is a water vibrio from Nanking.

13 strains of this type do not belong to any group, 8 were isolated from fæces, 4 from water and one is of unknown origin.

In the 4 last fermentative types, the strains not grouped form the majority. Only 4 strains within type III showed agglutinative

relationship: Nos 88, 303, 306 and 351, all agglutinated by Serum 303. Nos 88 and 303 are identical, but were sent to us from two different places, one from N. C. T. C. and one directly from the place of isolation at Calcutta. The other two strains also came from there. Nos 303 and 306 were isolated on Aug. 28th and on Aug. 29th, 1931, and strain No. 351 on March 3rd, 1931.

The remaining strains of these types do not show any relations of special interest.

In his publications Greig maintains, that in his material the vibrios isolated from water do not seem serologically related to those isolated from fæces, but this statement only expresses a relative condition, on account of the limited number of strains in his material. As is to be expected in a population who use river water for drinking and other purposes to a great extent, the present investigations of vibrios isolated from human fæces and from river water show that as far as agglutinative powers are concerned both kinds may be found in the same group. Greig's material undoubtedly did not show this because it was too small.

In addition to the properties of the strains agglutinable with cholera serum previously described, the classification also shows that vibrios which are non-agglutinable with cholera serum and which have been isolated from patients with diarrhoea, may often be found within certain groups, even though the localities where they have been isolated are far apart.

Although clinical examinations are outside the scope of the present work, information was asked for at Calcutta as regards the duration and termination of the disease in the cases from which vibrios in the present material were isolated, whether or not the vibrios had been agglutinable with cholera serum.

As regards "duration of the disease" — a vague form of expression — information was obtained in 119 cases; from 92 of them the vibrios isolated were "agglutinable", from 27 "non-agglutinable". In these two groups the duration of the disease was evenly distributed, from 2 to over 10 days; the mortality of these patients is shown in Table 26.

Table 26.

Outcome of disease	Non fatal	fatal
Cholera vibrio isolated	75	17
Cholera-like vibrio isolated	19	8

These figures, which show a surprisingly low mortality in the cases in which "true" cholera vibrios were found, indicate that the prognosis of the cases from which cholera-like vibrios were isolated is no better than that of those from which cholera vibrios were isolated. Of these 27 cholera-like vibrios, 24 were agglutinated by other sera. 20 of these 24 belong to the fermentative type II; 4 belong to type I: 294, 301, 309 and 340. The 3 non-agglutinated vibrios belong to the fermentative types II, IV and VI.

Thus there is no reason to suppose that any of these vibrios have been "true" cholera vibrios appearing in a non-agglutinable form.

d) *Investigation on the possible existence of O and H factors, analogous to those of the salmonella group.*

In order to determine the possible presence of thermostable O-antigen (somatic antigen) and thermolabile H-antigen (flagellar antigen), analogous to the antigens found in the salmonella group and a few other bacterical species, 2 of the strains with highest motility were chosen — N^{os} 64 and 211 — for the production of a number of sera; No. 211 had been isolated in March, 1933 and was 7 months old at the time the sera were made.

From each strain 5 sera were made in the usual manner. After 3 injections had been given to the rabbits, the titres were determined with both live and boiled cultures. Two more injections were then given at intervals of 5 days, without change of titre, and then the animals were totally bled.

The antigens for the production of the 5 sera were:

A) Emulsion in normal saline of a 20 hours' agar culture, boiled for 2 hours.

B) 20 hours' peptone-water culture, heated for 1 hour at 58 ° C..

C) Emulsion in normal saline of a 20 hours' agar culture, heated for 1 hour at 58 ° C..
D) Emulsion in "flagellar" suspension (see below) of a 20 hours' agar culture, heated for 1 hour at 58 ° C..
E) "flagellar suspension"; heated for 1 hour at 58 ° C..

In the account that follows these sera will be expressed by the letters A-E. The flagellar suspension was made by Gohar's method. A very dense suspension of vibrios which had been cultivated on agar was placed in a shaker for 2 hours; by this means the flagella are supposed to be separated from the bacillary bodies. After centrifuging the bodies a fluid is obtained which presumably contains numerous flagella, mixed with some bacterial bodies. Gohar found that such a suspension of flagella was preferable to an ordinary culture for investigations of H-factor.

In order to follow the technique of Gohar, the antigens for the production of the sera which are supposed specially to contain H have been heated for 1 hour at 58 ° C.. If heat differentiates somatic and flagellar antigen in a manner identical to that found in the salmonella group, serum A should correspond to a pure O-serum, the other sera should be H + O sera, D and E, however, being especially rich in H.

Agglutination with the sera and emulsions of 20 hours' agar cultures, partly live, partly boiled for 2 hours as antigens, and reading of the reactions after 1, 2 and 20 hours' incubation at 37 ° C. gave the results shown in Table 27.

The macroscopic appearance plays a great part in O and H agglutinations, and here the agglutinated particles were found to consist of finer and coarser particles, reminiscent in the last dilutions of the agglutination of B. abortus Bang; in the first tubes the particles were larger and collected at the bottom of the tube.

Thus, the appearance of the agglutinated particles does not indicate that we are dealing with two factors differing in quality.

When a boiled culture was used for antigen there was no agglutination after the first hour, whilst the tubes with live culture showed beginning agglutination with nearly all the sera. This is not due to a more rapid H-agglutination, as both Serum A_{64} and A_{211}, which are both theoretically O-sera without H-agglutinin, agglutin-

Table 27.

Antigen: Live emulsion of an agar culture of the homologous strain.

Reading	1 hour at 37°							2 hours at 37°							20 hours at 37°							
Serum	100	200	400	800	1600	3200	6400	100	200	400	800	1600	3200	6400	100	200	400	800	1600	3200	6400	Saline
A_{64}	2	2	2	1	0	0	0	3	3	3	2	1	0	0	3	3	3	3	3	1	0	0
B_{64}	0	0	0	0	0	0	0	2	2	2	0	0	0	0	3	3	3	3	3	1	0	0
C_{64}	2	2	2	0	0	0	0	3	3	3	2	1	0	0	3	3	3	3	3	1	0	0
D_{64}	2	2	2	1	0	0	0	3	3	3	3	1	0	0	3	3	3	3	3	2	1	0
E_{64}	0	0	0	0	0	0	0	2	2	2	2	1	0	0	3	3	3	3	3	3	1	0
A_{211}	2	2	2	1	0	0	0	3	3	3	2	1	0	0	3	3	3	3	3	2	0	0
B_{211}	0	0	0	0	0	0	0	2	2	2	1	0	0	0	3	3	3	3	3	1	0	0
C_{211}	2	2	2	0	0	0	0	3	3	3	3	2	0	0	3	3	3	3	1	0	0	0
D_{211}	2	2	2	1	0	0	0	3	3	3	2	1	0	0	3	3	3	3	3	1	0	0
E_{211}	2	2	2	1	0	0	0	3	3	3	1	0	0	0	3	3	3	3	3	1	0	0

Antigen: Emulsion of an agar culture of the homologous strain heated for 2 hours at 100° on water bath.

Reading	1 hour at 37°							2 hours at 37°							20 hours at 37°							
Serum	100	200	400	800	1600	3200	6400	100	200	400	800	1600	3200	6400	100	200	400	800	1600	3200	6400	Saline
A_{64}	0	0	0	0	0	0	0	1	0	0	0	0	0	0	3	3	3	3	1	0	0	0
B_{64}	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	3	3	3	1	0	0	0
C_{64}	0	0	0	0	0	0	0	1	1	0	0	0	0	0	3	3	3	3	1	0		0
D_{64}	0	0	0	0	0	0	0	1	1	0	0	0	0	0	3	3	3	3	3	2		0
E_{64}	0	0	0	0	0	0	0	1	1	0	0	0	0	0	3	3	3	3				0
A_{211}	0	0	0	0	0	0	0	1	0	0	0	0	0	0	3	3	3	3	3	1	0	0
B_{211}	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	3	2	1	0	0	0	0
C_{211}	0	0	0	0	0	0	0	1	1	0	0	0	0	0	3	3	3	3	2	0	0	0
D_{211}	0	0	0	0	0	0	0	1	1	0	0	0	0	0	3	3	3	3	2	0	0	0
E_{211}	0	0	0	0	0	0	0	1	0	0	0	0	0	0	3	3	3	3	2	0	0	0

Agglutination with theoretical H and H+O-sera (for further details see p. 136) and live and boiled cultures as antigen.

(The serum called A is a theoretically pure O-serum, produced from a culture that has been heated at 100° for 2 hours).

ated as much as the other sera during the first hour and the theoretically H-containing Serum E_{64} did not practically agglutinate at all within this period.

After two hours the tubes with boiled culture began to show agglutination, whilst the tubes with live cultures had now almost completed their agglutination.

After 20 hours all five sera showed that the titre reached with live culture was a little higher than the titre with boiled culture. This was also the case when the agglutinating serum was theoretically O-serum.

The conclusions to be formed from this first experiment must be as follows:

With the technique used here:

a) the appearance of the agglutinated particles does not permit to assume that reactions of different quality have taken place.

b) live vibrios are agglutinated more quickly than boiled vibrios, both by theoretically pure O-sera and by H + O-sera.

c) it is found, that if the theoretical H + O sera contain two agglutinins of different quality, their titres must be nearly equal.

Whilst it is not difficult to produce pure O-agglutination, as boiling of the antigens destroys any H-antigen that may be present, pure H-agglutination must be produced from an H + O Serum, after the O-agglutinin is removed by absorption. Table 28 gives the results of an experiment with such a serum from which the O-factor has been absorbed.

For the technique and experimental scheme, see p. 107 and p. 154.

Table 28.

Serum	Antigen	Serum dilutions 200	400	800	1600	3200	saline
$D_{64}/_{0}$	64, heated 100°, 2 h.	3	3	3	3	1	0
$D_{64}/_{0}$	64, live	3	3	3	3	1	0
$D_{64}/_{64}$, 100°	64, heated 100°, 2 h.	0	0	0	0	0	
$D_{64}/_{64}$, 100°	64, live	0	0	0	0	0	

Agglutination with a serum which theoretically is a pure H-serum, an H+O serum having been absorbed with boiled culture. As antigen for the agglutinations has been used partly live cultures — H+O-antigen — and partly boiled cultures — O-antigen.

This table shows that by absorption with theoretically pure O-antigen, the theoretically H + O serum was totally exhausted of the agglutinins which may be determined with this technique.

Thus the agglutination produced in these experiments must be due to reactions between thermostable antigens and their corresponding agglutinins.

Table 29 shows the results of a similar absorption experiment between Serum 94, produced according to the standard methods, and its homologous strain, after heating the absorbent antigen for 2 hours at 100 ° C..

Table 29.

Serum	Antigen	100	200	400	800	1600	3200	saline
94/0	94, live	3	3	3	3	2	1	0
94/0	94, heated 100^0, 2 h.	3	3	3	3	1	1	0
94/94, 100^0	94, heated 100^0, 2 h.	1	0	0	0	0	0	
94/94, 100^0	94, live	1	0	0	0	0	0	

Serum 94 absorbed with boiled culture. Agglutination with live and boiled culture.

These experiments show that with this technique Serum 94 also agglutinates with thermostable antigen, i. e., according to the present terminology, shows O-agglutination.

The immobilisation reaction of Arkwright-Mandelbaum.

1927 Arkwright demonstrated that whilst within the salmonella group, H serum paralyses the motility of strains containing the corresponding H antigen, the motility of the agglutinated particles remains intact after interaction between O-factors. This reaction has been further examined by Mandelbaum (1932). It was only natural to investigate whether the cholera vibrios showed similar reactions.

The examinations were carried out in hanging drop preparation, a small quantity of a 20 hours' agar culture being stirred directly into a drop of diluted serum. The drop was then examined microscopically for possible agglutination of the bacteria and any influence upon their motility.

As control I used both a culture emulsified in saline and one emulsified in a serum which had in no way shown antibodies corresponding to the culture examined.

The results are indicated in Table 30.

Table 30.

Strain 64.	Motility	Agglutination
Saline	+++	(+)
Serum 33, diluted 10 times	+++	(+)
Serum A_{64}, diluted 10 times	0	+++
Serum A_{64}, diluted 100 times	(0)	++

Note: Motility: +++ strongly motile
0 immediate cessation of motility
(0) cessation of motility, but not as instantanous as 0
Agglutination: +++ All elements agglutinated in large flakes
++ A few elements non-agglutinated
(+) Agglutination of only few elements

Microscopical examination of agglutination and motility in hanging drop preparations. Serum A_{64} has been produced by immunization of a rabbit with culture heated at 100° C. for 2 hours.

In this table it will be seen that Serum A_{64}, which has been produced from boiled vibrios, totally paralyses the motility of the vibrios. Further, the experiments given in Table 31 show that after absorption with culture heated at 100° C. for 2 hours, the theoretical H + O serum loses both its power of agglutination and of paralysing motility.

Table 31.

Strain 64.	Motility	Agglutination
Saline	+++	0
Serum D_{64}, non absorbed, diluted 80 times	0	+++
Serum D_{64}, absorbed with culture 64, heated at 100° for 2 h, diluted 80 times.	+++	(+)

Explanation of signatures, see Table 30.

Microscopical examination in hanging drop of agglutination and motility in a theoretical H+O serum and in the same serum after absorption with a culture heated at 100° C. for 2 hours.

Table 32.

Strain	Saline control	Serum 345 control		Serum Madsen		Serum A_{64} »O«-Serum		Serum D_{64} »H+O«-Serum	
	Aggl.	Motil.	Aggl.	Motil.	Aggl.	Motil.	Aggl.	Motil.	Aggl.
21	(+)	+++	(+)	+++	(+)	+++	(+)	+++	(+)
26	(+)	+++	(+)	+++	(+)	+++	(+)	+++	(+)
27	+++								
28	+	+++	(+)	+++	(+)	+++	(+)	+++	(+)
29	(+)	++	(+)	+++	(+)	+++	(+)	+++	(+)
32	+++								
33	(+)	+++	(+)	+++	+	++	(+)	++	(+)
34	(+)	+++	(+)	0	+++	0	+++	0	+++
35	(+)	++	(+)	++	(+)	++	(+)	++	(+)
36	0	0	0	0	++	0	++	0	+++
37	0	++	(+)	0	+++	0	++	0	+++
38	(+)	+++	0	0	+++	0	++	0	++
39	(+)	+++	(+)	+++	(+)	+++	(+)	+++	(+)
40	0	0	0	0	0	(+)	0	0	0
41	(+)	+++	(+)	0	+++	0	+++	0	+++

Note: Motility (Motil.): +++ all elements strongly motile
++ not quite so strong movements
0 none of the elements motile.

Agglutination (Aggl.): +++ All elements agglutinated, agglutination in large flakes
++ a few elements not agglutinated
(+) a few elements agglutinated
0 none of the elements agglutinated.

	Ser. 345	Ser. Madsen	Ser. A_{64}	Ser. D_{64}
Titre limit of dilutions used for examinations	3200	1600	3200	6400
	10	10	10	10

Microscopical examination of motility and agglutination in hanging drop preparations of a number of strains; in other quarters it has been supposed that they possessed a common H-antigen (except strain 35); the agglutination reactions have been carried out partly with the same sera otherwise used — serum Madsen — partly with specially produced »O« and »H+O« sera. Moreover, one vibrio serum — S 345 — has been used for control.

As mentioned on p. 103 Gardner in the autumn of 1933 considered that with a special technique he had proved that a series of strains might have a common H antigen, whilst only some of them had common O antigen.

In order to examine microscopically the agglutination reaction between some of these sera and strains, — and in order, amongst other things, to examine the reaction when serum containing the H antigen of a strain only was added — all the strains described by Gardner were examined with 4 sera: the serum used by Gardner, called Serum Madsen, Serum A_{64}, Serum D_{64} and, as a control, Serum 345. The results of these experiments are given in Table 32.

This shows that in none of the cases in which the serum according to Gardner is supposed to contain the H agglutinin of the strains only — the strains 21—26—27—29—33—39—40 — did agglutination or paralysis of motility occur, in spite of the fact that the examinations were carried out in small dilutions of serum. In the cases in which Gardner found common O antigen, the experiments showed agglutination and cessation of motility. This at any rate does not indicate that the reactions are analogous to the reactions of the salmonella group.

The experiments described above, carried out in order to prove the existence of O and H antigens, analogous to those of the salmonella group have shown that the reactions seem to be due only to a reaction between a thermostable antigen and its agglutinins. As this technique is fairly similar to that used in general diagnosis, this fact is in favour of the agglutination reaction read in common diagnostic examinations being caused by interactions with thermostable antigen.

But the question arises: Will another agglutination technique show different results?

It is impossible to answer this beforehand.

As mentioned above, Douglas by using formolized bouillon culture as antigen for the agglutination reaction, found that a vibrio which had previously been called non-agglutinable with ordinary cholera serum in his experiments was agglutinated by a cholera serum in a dilution 4 times the titre of the homologous strain. Douglas himself believes that it is a non-specific reaction, as the strain in question

cannot exhaust serum of agglutinin against cholera vibrios. He did not mention, whether it could exhaust cholera serum against itself. As Gardner also described divergent agglutination results in cases where formolized bouillon cultures had been used for the agglutination reactions, it will be seen that question of the technique itself has again become of importance.

Gardner examined 18 strains of vibrios. 17 of them were agglutinated by a cholera serum if formolized bouillon culture was used as antigen and the reaction read after 20 hours' incubation at 52° C.. If, on the other hand, an emulsion of boiled agar culture was used for the agglutinations, 8 strains were non-agglutinable with the serum used. Gardner interpreted this as the reaction with formolized bouillon culture being an H-agglutination (perhaps H + O); the reaction with boiled culture was supposed to be a pure O-agglutination. He states that absorption experiments with boiled culture confirm this supposition. The expressions O and H are here used in analogous manner to the terms used within the salmonella group.

I have repeated Gardner's experiments and obtained the same results, and also carried out various supplementary examinations with Gardner's technique.

If these phenomena form a parallel to the salmonella group as regards the properties of the O and H factors, the following reactions are to be expected:

1) The H-reaction should cause loose, floccular agglutination, which is easily destroyed by shaking, whilst the O-reaction should cause fine, compact granules.

2) H-agglutination must appear quickly — after ½ hour —, O-agglutination more slowly, after 4—20 hours.

3) If the motility in bouillon culture and in culture emulsified in normal saline is the same, it is to be expected that the reactions will be produced with both kinds of culture. Moreover, addition of formalin should inhibit O-agglutination, but stimulate H-agglutination.

4) H-antigen must be destroyed by boiling, in contrast to O-antigen which must be thermostable.

5) H-agglutinin must be thermostable, in contrast to O-agglutinin, which is destroyed by heat.

And finally, although it is somewhat outside the limits of the above stipulations:

6) According to the Arkwright-Mandelbaum reaction H-serum must paralyse the motility of the bacteria, in contrast to O-serum, which should leave the motility unaffected.

In regard to the first requirement, I did not find the appearance of the agglutination tube, — in the cases which according to Gardner should be H-agglutinations — sufficiently characteristic for qualitative evaluation. The precipitation was of a more or less

Table 33.

Serum D_{64}		1 hour at 52°						2 hours at 52°						20 hours at 52°						
		Serum dilutions																		
		200	400	800	1600	3200	6400	200	400	800	1600	3200	6400	200	400	800	1600	3200	6400	saline
form. bouil. cul.	39	0	0	0	0	0	0	0	0	0	0	0	0	3	3	3	2	1	0	0
— —	64	3	3	2	1	0	0	3	3	3	2	0	0	3	3	3	3	1	0	0
live emulsion	39	0	0	0	0	0	0	0	0	0	0	0	0	(1)	(1)	(1)	(1)	0	0	0
— —	64	3	3	2	1	0	0	3	3	3	3	1	0	3	3	3	3	2	1	0
form. emuls.	39	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	0	0
— —	64	3	3	3	2	0	0	3	3	3	3	1	0	3	3	3	3	3	1	0

Serum 39		Serum dilutions																		
		200	400	800	1600	3200	6400	200	400	800	1600	3200	6400	200	400	800	1600	3200	6400	saline
form. bouil. cul.	39	3	3	3	3	2	1	3	3	3	3	3	2	3	3	3	3	3	3	0
— —	64	0	0	0	0	0	0	0	0	0	0	0	0	3	3	3	3	3	3	0
live emulsion	39	3	3	3	3	3	2	3	3	3	3	3	2	3	3	3	3	3	3	0
— —	64	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
form. emuls.	39	3	3	3	3	3	2	3	3	3	3	3	3	3	3	3	3	3	3	0
— —	64	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Cross agglutination of the strains 39 and 64 — mutual thermolabile antigen, but not mutual thermostable antigen; live emulsion of agar cultures have been partly used as antigen, partly formolized emulsions and partly formolized bouillon culture. The agglutination reactions have been read after 1, 2 and 20 hours' heating at 52° C. (water-bath).

indefinable appearance, some strains giving a finely granulated precipitation, some a more floccular in all tubes of the titration. I must also mention, that the reading was difficult, as in some cases it was almost impossible to determine whether there was any agglutination or not. Reading with a magnifying glass often made a determination impossible, as upon growth in bouillon the cultures often show very fine spontaneous agglutination.

Table 33 indicates the time interval before the so-called H-agglutination appeared.

In this case, the "H-agglutination" is seen to be a reaction appearing comparatively later than the "O-agglutination". This agrees with Gardner's statement that readings should be taken at 20 hours; but it is quite contrary to what takes place within the group of salmonella.

In regard to the third requirement, the examination of the influence of the condition of the antigen, a strange phenomenon was observed. The following experiment illustrates this:

2 strains, N^{os} 39 and 64, and sera produced from these strains, were used for cross agglutination, using formolized bouillon culture and live bacterial emulsion as antigen. The reactions were read after 20 hours, and were carried out both at 37° C. and at 52° C..

As seen in table 34, strain 64 was found to be non-agglutinable with Serum 39 if live emulsion were used as antigen, but it was strongly agglutinable if formolized bouillon culture were used and the reaction was carried out at 52° C.. Formolized bouillon culture at 37° C. caused a suggestion of an agglutination only, equally strong in all tubes.

Strain 64 is agglutinated strongly by Serum 64 (for the experiments D_{64}, described on p. 137 has been used), independent of the technique used.

An emulsion of strain 39 was slightly agglutinable in the first two tubes when the reaction was carried out at 52° C.; at 37° C. there was a very slight precipitation in the otherwise quite opaque and non-agglutinated first four tubes. This finding, however, was not constant when the experiments were repeated. If, however, agglutination was carried out with formolized bouillon culture, strong agglutination was seen at 52° C., varying agglutination at 37° C.. Lastly, the same experiment has been carried out with one of the

Table 34.

Serum D_{64}

	37° Serum dilutions						52° Serum dilutions					
	400	800	1600	3200	6400	saline	400	800	1600	3200	6400	saline
Strain	Antigen: formolized bouillon culture											
39	2	2	3	3	2	1	3	3	3	3	3	0
64	3	3	3	3	3	0	3	3	3	3	3	0
	Antigen: live emulsion of agar culture											
39	1	1	1	1	1	0	2	1	0	0	0	0
64	3	3	3	3	2	0	3	3	3	3	2	0

Serum 39.

	37° Serum dilutions						52° Serum dilutions					
	400	800	1600	3200	6400	saline	400	800	1600	3200	6400	saline
Strain	Antigen: formolized bouillon culture											
39	3	3	3	3	3	1	3	3	3	3	3	0
64	1	1	1	1		0	3	3	2	2	1	0
	Antigen: live emulsion of agar culture											
39	3	3	3	3	2	0	3	3	3	2	1	0
64	(1)	0	0	0	0	0	0	0	0	0	0	0

Serum Madsen.

	37° Serum dilutions						52° Serum dilutions					
	50	100	200	400	800	saline	50	100	200	400	800	saline
Strain	Antigen: formolized bouillon culture											
39	3	2	3	3	3	(1)	3	3	3	3	3	(1)
64	3	3		3	3	0	3	3	3	3	3	0
	Antigen: live emulsion of agar culture											
39	1	0	0	0	0	0	1	1	0	0	0	0
64	3	3	3	3	2	(1)	3	3	3	2	(1)	0

Investigation of the influence of the condition of the antigen: formolized bouillon culture or live emulsion of agar culture, upon the occurrence of the agglutination; also investigation of the effect of the temperature: 37° or 52° C..

sera used by Gardner, Serum Madsen: the results were here analogous to those obtained with Serum 64.

The fact that an emulsion of live, motile vibrios is not agglutinated although a formolized bouillon culture is strongly agglutinated shows the decisive influence of the technique upon the result of a reaction.

Table 33 further shows that addition of formalin to an agar culture does not influence the agglutinability to any degree worth mentioning. Table 32 shows that the O-agglutination is not inhibited by the formalin, the titre of Serum A_{64} being unaltered, 1600, when formolized bouillon culture is used as antigen.

Requirement 4) deals with the main properties of the O and H antigens of the salmonella group: O antigens can stand boiling for as much as 2 hours, whilst the H antigens are quickly destroyed by boiling, but are stable after heating to 60 ° C.. A number of experiments were carried out in order to note the reactions of the vibrios with Gardner's agglutination technique.

The agglutination of the strains 39 and 64 was examined against a serum produced from boiled vibrios of the strain 64 — Serum A_{64} — and against the Serum D_{64} previously described (p. 137).

Table 35.

	Serum A_{64}						Serum D_{64}						
	Serum dilutions						Serum dilutions						
	200	400	800	1600	3200	saline	400	800	1600	3200	6400	12800	saline
St. 39	0	0	0	0	0	0	3	3	3	3	3	1	0
— 64	3	3	3	1	0	0	3	3	3	3	3	3	0

Antigen: formolized bouillon culture.

Agglutination with theoretical »O« serum — A_{64} — and theoretical »H+O« serum — D_{64}.

This showed that strain 39 was non-agglutinable with the theoretical O-serum, but, as previously, agglutinable with Serum D_{64}. This shows that the agglutination of Strain 39 with Serum D_{64} is due to an agglutinin in the serum which is antibody to a thermolabile antigen in the strain 64.

Agglutination tests using the Sera 39 and D_{64} — Serum 39 having been produced as described on p. 105 — and formolized and boiled bouillon cultures of the strains 39 and 64 as antigens, showed reactions which seemed to confirm the supposition of a common thermolabile and special thermostable factors in these strains. Whilst the formolized bouillon cultures of both strains were agglutinated by both sera, the boiled cultures were only agglutinated by their homologous sera. The experimental results are shown in the table of the following experiments, Table 36.

Lastly, absorption tests were carried out with the two Sera 39 and D_{64} and the strains N^{os} 39 and 64, bacterial emulsions in normal saline being used after heating either for 15 minutes at 55 ° C., or for 2 hours at 100 ° C.. The results are shown in Table 36.

These experiments showed that boiled culture of strain 64 cannot absorb agglutinins from Serum 39, in contrast to the culture that had only been heated to 55 ° C., which can remove the agglutinin for 64, but not — at any rate not all — for 39. On the contrary, boiled culture 39 does not remove agglutinin from Serum 64, whilst culture that has only been slightly heated removes agglutinin for 39, but at any rate not all agglutinin for 64. Agglutination with boiled bouillon culture and these absorbed sera give the results which might be expected.

The limits of the agglutination titres with formolized and boiled cultures do not differ sufficiently to make it possible definitely to state more than that the titre of "H" agglutinins is of about the same value as that of "O" agglutinins.

All the boiled bouillon cultures have shown a slight degree of spontaneous agglutinability, but not more than it has been possible macroscopically to determine the quantitative differences which appeared in tubes where agglutination took place.

In order to observe how rapidly the thermolabile antigen is destroyed, bouillon culture from strain 39 was heated at 100 ° C. for 15, 30 and 60 minutes, and after addition of formol it was agglutinated with S. 64 and S. 39. The result given in Table 37, showed that heating even for 15 minutes at 100 ° C. rendered strain 39 non-agglutinable with Serum 64, whilst its agglutinability towards Serum 39 was not destroyed even after heating for 60 minutes at 100 ° C.

Table 36.

Serum	absorb. with strain	agglutin.	Form. bouill. cult. Serum dilutions						Boiled bouill. cult. Serum dilutions					
			400	800	1600	3200	6400	sal.	400	800	1600	3200	6400	sal.
39		39	3	3	3	3	1	0	3	3	3	3	2	1
39		64	3	3	3	2	0	0	1	1	1	1	1	1
39	64, heat. 15 min. 55°	39	3	3	3	1	0	0	3	3	3	3	1	1
39	64, — — —	64	0	0	0	0	0	0	1	1	1	1	1	1
39	64, heat. 2 h. 100°	39	3	3	3	3	2	0	3	3	3	3	3	1
39	64, — — —	64	3	3	3	3	1	0	1	1	1	1	1	1
39	39, heat. 15 min. 55°	39	0	0	0	0	0	0	1	1	1	1	1	1
39	39 — — —	64	0	0	0	0	0	0	1	1	1	1	1	1
39	39, heat. 2 h. 100°	39	3	3	2	1	0	0	1	1	1	1	1	1
39	39, — — —	64	3	3	3	2	1	0	1	1	1	1	1	1
64		39	3	3	3	2	1	0	1	1	1	1	1	1
64		64	3	3	3	3	2	0	3	3	2	1	1	1
64	39, heat. 15 min. 55°	39	0	0	0	0	0	0	1	1	1	1	1	1
64	39, — — —	64	3	3	2	0	0	0	3	3	3	2	1	1
64	39, heat. 2 h. 100°	39	3	3	3	2	0	0	1	1	1	1	1	1
64	39, — — —	64	3	3	3	2	1	0	3	3	3	2	1	1
64	64, heat. 15 min. 55°	39	0	0	0	0	0	0						
64	64, — —	64	0	0	0	0	0	0						
64	64, heat. 2 h. 100°	39	3	3	2	1	(1)	0	1	1	1	1	1	1
64	64, — — —	64	3	3	3	2	1	0	1	1	1	1	1	1

Absorption of serum 39 and serum D_{64} with non-boiled and boiled emulsions of agar cultures of strains 39 and 64; then agglutination of formolized and boiled bouillon cultures of the same two strains.

Table 37.

Strain 39 bouillon cult.	S. 39 Serum dilutions 400	800	1600	3200	6400	12800	S. D_{64} Serum dilutions 400	800	1600	3200	6400	12800	saline
non-heated.	3	3	3	3	3	2	3	3	3	3	3	2	0
heat. 100° 15 min	3	3	3	3	2	2	(1)	0	0	0	0	0	0
— 100° 30 min.	3	3	3	3	2	2	0	0	0	0	0	0	0
— 100° 60 min.	3	3	3	3	2	2	0	0	0	0	0	0	0

Agglutination of bouillon culture of strain No. 39, non-heated and heated different periods at 100°. The sera used have been serum 39, theoretically containing »H+O« agglutinin against strain 39, and D_{64}, containing only the »H« agglutinin against the strain.

Thus it is not possible to determine the thermolabile antigen in bouillon culture which has been heated for 15 minutes at 100° C..

The present experiments have proved definitely that the strains Nos 39 and 64 contain a common thermolabile antigen and a thermostable, groupspecific antigen.

But, moreover, the experiments showed something else by no means unimportant.

Mixture of Serum 39, e. g., and an emulsion of agar culture of strain No. 64 does not cause visible flocculation, but nevertheless the first step in agglutination, the fixation between antigen and agglutinin, takes place. As already stated, all the absorption experiments have been carried out with emulsions of agar culture.

These experiments have confirmed the earlier experience, that agglutination with live bacterial emulsions may be compared with an O-agglutination. They have also confirmed, what has been proved amongst others by Gardner, that it is possible, by a special technique, to prove in vibrios two different antigens, which may be separated by heat.

In regard to the differentiating ability of heat on the antigenic factors, a thermolabile and a thermostable antigen have been found here, as is the case within the salmonella group.

In regard to the 5th requirement, the influence of heat upon the agglutination reaction, it was found that the agglutinins corresponding to the thermolabile antigens were also thermolabile,

whilst the agglutinins of the thermostable antigens were more thermostable. This was demonstrated in the following experiments:

Serum 64 was heated in concentration 1/160 for 5, 10, 15 and 20 minutes, and agglutination was carried out with these sera and formolized bouillon culture of strain 39 — representing only the thermolabile antigen corresponding to this serum — and formolized bouillon culture from strain 64 — representing both thermolabile and thermostable antigens; these reactions showed, as seen in Table 38, that the agglutinin corresponding to the thermolabile antigen is thermolabile in itself, as Serum D_{64} had after 10 minutes practically lost its agglutinating power towards strain 39, contrary to what is the case towards strain 64, the agglutinating power here only being relatively slightly influenced even after double that time.

Table 38.

Culture	64 (form. bouillon cult.) Serum dilutions						39 (form. bouillon cult.) Serum dilutions						
Serum D_{64}	400	800	1600	3200	6400	12800	400	800	1600	3200	6400	12800	saline
non-heated.	3	3	3	3	3	1	3	3	3	3	3	1	0
heat. 70° 5 min.	3	3	3	3	1	1	2	2	1	1	1	1	
heat. 70° 10 min.	3	3	3	2	1	0	1	0	0	0	0	0	
heat. 70° 15 min.	3	3	3	1	0	0	2	1	0	0	0	0	
heat. 70° 20 min.	3	3	3	2	0	0	2	0	0	0	0	0	

Examination of agglutination with serum D_{64} — a theoretical »H+O« serum — after heating for different periods at 70°, the agglutination being examined with formolized bouillon cultures of the strains 64 and 39.

In this case thermostable antigen was found to have relative thermostable agglutinin and the thermolabile antigen to have relative thermolabile agglutinin, contrary to what is the case in the salmonella group, in which thermostable antigens form thermolabile agglutinins, and thermolabile antigens form thermostable agglutinins.

The 6th requirement indicated as characteristic of the H and O sera of the salmonella group was the immobilisation reaction of Arkwright-Mandelbaum. As already mentioned this really lies out-

side the limits of the reactions used here for determining O and H factors, the determination of the H factors being based upon examination of formolized bouillon cultures and not on that of live cultures; but, on the other hand, the reactions given by the vibrios as far as this behaviour is concerned, and discussed in detail on p. 140, are so strange when compared with the reactions of the salmonella group, that it would seem wrong not to mention them here.

Summarizing these somewhat preliminary experiments, on the presence of two different antigenic factors in the Vibrio Choleræ and closely related vibrios analogous to the antigens found in the salmonella group, the following statement must be made:

The macroscopically visible precipitation in normal saline emulsion of live vibrios mixed with agglutinating serum seems to be caused by a reaction with thermostable antigen.

Sera which do not cause precipitation in emulsions of live cultures of certain strains sometimes cause slow precipitation with formolized bouillon cultures. The precipitated particles may be looser or more compact. The reaction is often difficult to read, as it may be difficult to determine whether there is any reaction at all. The reaction which only appears in formolized bouillon cultures is due to a thermolabile antigen.

The agglutinin corresponding to a thermostable antigen is thermostable itself compared with the agglutinin corresponding to the thermolabile antigen.

Microscopical examination carried out shortly after preparing an emulsion of live, motile vibrios in a serum containing only agglutinins towards the thermolabile antigen, does not show any influence upon the vibrios, either as regard their motility or agglutination. After emulsion in a serum which only contains agglutinin corresponding to the thermostable antigens, the vibrios become agglutinated and non-motile.

Thus the thermolabile and thermostable antigens which have been found in the cholera vibrios differ in several ways from the thermolabile and thermostable factors of the salmonella group.

When a vibrio in literature or elsewhere has previously been described as agglutinable or non-agglutinable with cholera serum, it concerned (except in a very few cases) reactions carried out with live vibrios emulsified in normal saline. But from the above experi-

ments with both formolized bouillon cultures and emulsions in normal saline, we have learnt that the mere finding of agglutination or non-agglutination with cholera serum does not suffice. The thecnique used for the agglutination reactions must also be indicated.

e) *Absorption experiments.*

The absorption reactions form a very important part of the examinations of the antigenic factors of a bacterium. Only by means of these reactions can one prove the possible identity of two bacteria. If we call the two bacteria, the antigenic composition of which we wish to investigate, A and B, an absorption reaction may be carried out in the following manner:

Sera are prepared from both strains; absorption and agglutination between these sera and the two strains are carried out according to the following scheme:

Serum	antigen	dilutions of serum
Ser A/O	A	
—	B	
Ser A/A	A	
Ser A/B	B	
—	A	
Ser B/O	B	
—	A	
Ser B/B	B	
Ser B/A	A	
—	B	

In the first column the serum used for the agglutination is stated; A/B indicates that serum A has been absorbed by strain B, the letter above the line giving the serum used for absorption, the letter below the strain used for absorbing the serum.

The figure O below the line means that the serum has not been absorbed in the experiment in question.

The technique of the absorption experiments has been described on p. 107.

In the second column the antigen for the agglutination reactions is indicated.

A total of 10 agglutinations tests are necessary for a complete examination. The agglutination tests are divided in halves, one including the examinations to find whether all the antigen of A is found in B, the other those to find whether all the antigen of B is present in A.

The two first agglutination tests with non-absorbed serum A and the antigens A and B show how highly the serum can agglutinate the strains. The third agglutination test shows whether all the agglutinin present in serum A is removed by absorption with A with the technique used here. The two next agglutination tests show the agglutinogenic power of serum A after the agglutinins fixed by B have been removed. The agglutination test with B controls whether the absorption of the B agglutinating factors was complete. The agglutination test with A shows whether serum A possessed other agglutinins than those removed by B. If agglutination takes place here, this means that the strain A contains one or several antigen factors which are not present in B. If no agglutination takes place, it means that all the antigenic factors of strain A are found in strain B.

In the same manner the next 5 agglutination tests show whether all the antigens of strain B are present in strain A.

Only if such a cross absorption test, i. e., examination of both strains towards both sera has been carried out, is it possible to determine whether two strains are identical or not.

The general value of the present absorption experiments is limited, because the highest serum concentration examined corresponts to 16/T (T = the titre). Thus, this technique will not show factors with so small agglutinogenic power that in this dilution the corresponding agglutinins are present in such small quantities that visible and strong reactions do not occur. Larger quantities of serum in the first test tube might sometimes form such large concentrations of agglutinins that unspecific reactions will appear. Further, the use of serum dilutions in proportion to the titre form better comparative material in different sera than absolute values for titration.

Besides the absorption experiments mentioned in the discussion of Table 16, a few other experiments have been carried out, which will now be described.

On p. 116 it was mentioned that the agglutinins of sera 8 and 94 most likely differed as to their quality. Cross absorption showed, in fact, (see Table 39) that whilst all the antigenic factors of strain 8 were present in strain 94, strain 94 being able to exhaust serum 8 of agglutinins for strain 8, strain 94 contained one more antigen group, as strain 8 could not exhaust serum 94 of all agglutinin for strain 94.

Table 39.

		Serum dilutions					
Serum	Antigen	100	200	400	800	1600	saline
8/0	8	3	3	3	2	1	0
8/0	94	3	3	3	2	0	0
8/8	8	0	0	0	0	0	0
8/94	94	0	0	0	0	0	0
8/94	8	2 (0)*)	0	0	0	0	0
		200	800	1600	3200	6400	
94/0	94	3	3	3	3	2	
94/0	8	3	2	1	0	0	
94/94	94	0	0	0	0	0	
94/8	8	0	0	0	0	0	
94/8	94	3	3	3	2	0	

Cross absorption between the strains 8 and 94.

*) By repeating the test no agglutination appeared in this tube.

If we call the antigenic group of strain 8 A, we may, for instance, call the group of strain 94 AB. As none of the strains in the total material were agglutinable with strain 94 without also being agglutinable with strain 8, it is clear that in the material examined here, the factor B has only appeared in combination with the factor A.

As the strain 94 was found to be constantly hæmolytic, the relationship was investigated between serum 94 and the rest of the strains that were both agglutinable with cholera serum and hæmolytic, bearing in mind the possibility that the factor B particularly might be found in the strains of this serological group which had at some time or other appeared as hæmolytic.

Absorption of serum 94 with these strains showed the results indicated in Table 40.

Table 40.

Serum	absorbed by strain	agglutin. with strain	serum dilutions 200	400	800	1600	3200	6400	saline
94		94	3	3	3	3	2	1	0
94	94	94	0	0	0	0	0	0	
94		44	3	3	3	1	0	0	0
94	44	44	0	0	0	0	0	0	
94	44	94	3	3	3	2	1	0	
94		45	3	2	1	0	0	0	0
94	45	45	0	0	0	0	0	0	
94	45	94	3	3	3	2	1	0	
94		49	3	3	3	1	0	0	0
94	49	49	0	0	0	0	0	0	
94	49	94	3	3	3	3	2	0	
94		54	3	2	0	0	0	0	0
94	54	54	0	0	0	0	0	0	
94	54	94	3	3	3	3	2	0	
94		71	3	3	3	1	0	0	0
94	71	71	0	0	0	0	0	0	
94	71	94	3	3	3	3	0	0	
94		100	3	3	3	3	1	0	0
94	100	100	0	0	0	0	0	0	
94	100	94	3	3	3	3	1	0	
94		101	3	3	3	3	2	0	0
94	101	101	0	0	0	0	0	0	
94	101	94	0	0	0	0	0	0	
94		103	3	3	3	3	2	0	0
94	103	103	0	0	0	0	0	0	
94	103	94	0	0	0	0	0	0	

(cont. next page.)

Serum	absorbed by strain	agglutin. with strain	serum dilutions 200	400	800	1600	3200	6400	saline
94		106	3	2	0	0	0	0	0
94	106	106	0	0	0	0	0	0	
94	106	94	(1)	0	0	0	0	0	
94		113	3	3	3	3	3	0	0
94	113	113	0	0	0	0	0	0	
94	113	94	0	0	0	0	0	0	
94		258	3	3	3	1	0	0	0
94	258	258	0	0	0	0	0	0	
94	258	94	2	1	0	0	0	0	
94		369	3	3	3	1	0	0	0
94	369	369	0	0	0	0	0	0	
94	369	94	3	3	3	3	1	0	

Examination of the power of absorption of the hæmolytic strains agglutinable with cholera serum, against serum 94.

It will be seen that the strains 101, 103, 106 and 113 completely exhaust serum 94, and therefore they must contain the factors A and B. The strain 258 causes nearly complete exhaustion; as it is agglutinated by serum 8 it contains the factor A, and as seen from this experiment, it very probably also contains B; but it has not caused total absorption.

In contrast to this the strains 44, 45, 49, 54, 71, 100 and 369 can influence the agglutinogenic power of serum 94 towards strain 94 only slightly. As all these strains are agglutinable with strain 8 and thus contain factor A, this probably means that they do not contain B. Whether or not the strains contain the total factor group A may be gathered after absorption of serum 8 with the strains. As seen in Table 41, all the strains except one could absorb the agglutinins from serum 8 almost completely (the exception being strain 100). I have not felt justified in attaching any importance to the weak reactions in the first dilution tube.

The fact that strain 100 cannot absorb serum 8 seems to indicate that group A consists of more than one factor, only part of the group being present in strain 100.

Table 41.

Serum	absorbed by strain	agglutin. with strain	Serum dilutions 100	200	400	800	1600	saline
8		8	3	3	3	3	1	0
8	8	8	1	0	0	0	0	
8		44	3	3	3	2	1	0
8	44	44	0	0	0	0	0	
8	44	8	1	0	0	0	0	
8		45	3	3	3	2	0	0
8	45	45	0	0	0	0	0	
8	45	8	1	0	0	0	0	
8		49	3	3	3	2	0	0
8	49	49	0	0	0	0	0	
8	49	8	1	0	0	0	0	
8		54	3	3	3	2	1	0
8	54	54	0	0	0	0	0	
8	54	8	2	0	0	0	0	
8		71	3	3	3	2	0	0
8	71	71	0	0	0	0	0	
8	71	8	1	1	0	0	0	
8		100	3	3	3	2	0	0
8	100	100	0	0	0	0	0	
8	100	8	3	3	3	1	0	
8		258	3	3	3	2	0	0
8	258	258	0	0	0	0	0	
8	258	8	(1)	(1)	(1)	0	0	

Explanation see p. 158.

It has now been shown that the factor B is not connected with all the hæmolytic strains of this group, but the possibility still remains that it is only present in the constantly hæmolytic strains. In order to find whether this was the case or not, and to determine all the strains in the present material containing the factor B, all the strains

agglutinable with serum 8 were agglutinated with a serum that contained agglutinin for the factor B but not for the factor A.

This serum was produced by absorbing serum 94, which contains agglutinin for both factors A and B, with strain 8, which can only absorb agglutinin for the factor A.

Agglutination test on a microscopic slide were carried out with this serum and all the strains that were agglutinable with serum 8; serum was used in the dilution 1/100, as preliminary experiments with strain 8 and strain 94 had shown that with these strains the dilution 1/100 caused immediate and strong agglutination. The results are given in Table 42.

Table 42.

Agglutination on microscopic slide using serum 94 non-absorbed and serum 94 absorbed with strain No. 8.

The examinations include all the strains that are agglutinable with serum 8.

Strain	saline	ser. 94/0	ser. 94/8	Strain	saline	ser. 94/0	ser. 94/8
1.	++	++	++	25.	0	+++	+++
2.	0	+++	0	32.	+	+++	++
3.	++	++	++	34.	+	+++	+
4.	+	++	+	36.	0	++	0
5.	++	+++	++	37.	0	+++	0
8.	0	+++	0	38.	0	+++	0
9.	0	+++	+++	41.	+	+++	++
10.	0	+++	+++	42.	+	++	+
11.	+	+++	++	43.	(+)	+++	(+)
12.	0	+++	0	44.	0	+++	0
13.	0	+++	(+)	45.	0	+++	0
14.	(+)	+++	(+)	46.	+	+++	+
15.	++			47.	0	+++	+++
16.	0	+	0	48.	0	++	0
17.	+	++	+	49.	0	+++	(+)
18.	++			50.	(+)	+++	+
19.	+	+++	+	51.	+++		
20.	+			52.	0	+++	0
22.	+			53.	0	+++	0
23.	0	+++	+++	55.	0	+++	0

Strain	saline	Ser. 94/0	Ser. 94/8
56.	0	+++	0
57.	0	+++	0
58.	0	+++	0
59.	+++		
62.	0	+++	+++
64.	0	+++	0
65.	+++		
66.	+	+++	+
67.	0	+++	0
71.	0	+++	0
74.	(+)	+++	(+)
80.	0	+++	0
87.	0	+++	0
90.	(+)	+++	(+)
91.	(+)	+++	(+)
94.	0	+++	+++
95.	(+)	++	(+)
96.	+	++	+
97.	0	+++	0
98.	++		
99.	++		
100.	0	+++	0
101.	(+)	+++	+++
103.	0	+++	+++
106.	0	+++	0
107.	(+)	+++	(+)
108.	0	++	0
110.	0	+++	+++
113.	0	+++	+++
114.	0	+++	+++
115.	++		
116.	+	+++	+++
117.	(+)	+++	+++
118.	0	+++	+++
119.	0	+++	0
121.	0	+++	0
122.	(+)	+++	(+)

Strain	saline	Ser. 94/0	Ser. 94/8
123.	0	+++	0
124.	0	+++	0
126.	+	+++	+
127.	0	+++	0
128.	0	+++	0
129.	0	+++	0
130.	(+)	+++	(+)
131.	0	+++	0
132.	(+)	++	(+)
133.	(+)	++	(+)
134.	++		
135.	(+)	+++	(+)
136.	0	+++	0
137.	(+)	+++	(+)
139.	(+)	++	(+)
140.	+	+++	+
142.	(+)	+++	+
143.	0	+++	0
144.	0	+++	+++
146.	0	+++	++
147.	0	++	(+)
148.	0	+++	+
149.	(+)	+++	(+)
150.	0	+++	++
151.	0	+++	+
152.	0	+++	++
153.	(+)	+++	+
154.	(+)	+++	+
155.	(+)	+++	(+)
156.	0	+++	0
157.	0	+++	0
158.	0	+++	+++
160.	0	+++	0
161.	0	+++	+++
162.	+		
163.	0	+++	0
164.	0	+++	0

Strain	saline	Ser. 94/0	Ser. 94/8	Strain	saline	Ser. 94/0	Ser. 94/8 uncertain reaction
165.	0	+++	++	208.	0	+++	
166.	0	+++	0	209.	0	+++	0
168.	0	+++	0	210.	0	+++	0
169.	0	+++	0	211.	0	+++	0
170.	0	+++	0	212.	0	+++	0
171.	0	+++	0	213.	0	+++	0
172.	0	+++	0	214.	0	+++	0
173.	0	+++	+++	215.	0	+++	0
174.	0	+++	+++	216.	0	+++	0
175.	0	+++	+	217.	0	+++	0
176.	0	+++	+++	218.	+++		
177.	0	+++	+++	219.	0	+++	0
179.	0	+++	+++	221.	0	+++	0
180.	0	+++	0	222.	0	+++	0
181.	0	+++	+++	223.	0	+++	0
184.	0	+++	+	224.	0	+++	0
185.	0	+++	+++	225.	0	+++	0
186.	0	+++	0	226.	0	+++	0
187.	0	+++	++	227.	0	+++	0
188.	0	+++	0	228.	0	++	0
189.	0	+++	0	229.	0	+++	0
190.	0	+++	+++	230.	0	+++	0
191.	0	+++	0	231.	0	+++	0
192.	0	+++	0	232.	0	+++	0
193.	0	+++	0	233.	0	+++	0
194.	0	+++	+++	235.	0	+++	0
195.	0	+++	0	236.	0	+++	0
196.	0	+++	0	237.	0	+++	0
197.	0	+++	0	241.	0	+++	0
198.	0	+++	0	242.	0	+++	0
199.	0	+++	0	244.	0	+++	0
200.	0	+++	0	245.	0	0	0
201.	0	+++	0	248.	0	+++	0
202.	0	+++	0	253.	+++		
203.	0	+++	0	254.	0	0	0
206.	0	+++	0	256.	0	+++	0
207.	0	+	0	257.	0	+++	0

Strain	saline	Ser. 94/0	Ser. 94/8	Strain	saline	Ser. 94/0	Ser. 94/8
258.	(+)	+++	+++	368.	0	+++	0
261.	0	+++	0	369.	0	+++	0
262.	0	+++	0	370.	0	+++	0
263.	0	+++	0	371.	0	+++	0
264.	0	+++	0	372.	0	+++	0
266.	0	(+)	0	373.	0	+++	0
268.	0	0	0	374.	0	+++	0
275.	0	+++	0	375.	0	+++	0
276.	0	+++	0	376.	0	+++	0
279.	0	+++	0	378.	0	+++	0
280.	0	+++	0	379.	0	+++	0
281.	0	+++	0	385.	0	+++	0
287.	0	+++	0	390.	0	+++	0
289.	0	+++	0	391.	0	+++	0
290.	0	+++	0	392.	0	+++	0
322.	0	+++	0	397.	0	+++	0
359.	0	+++	0	398.	0	+++	0
360.	0	+++	0	399.	0	+++	0
361.	0	+++	0	400.	0	+++	0
362.	0	+++	0	401.	0	+++	0
363.	0	+++	0	402.	0	+++	0
364.	0	+++	0	403.	0	+++	0
365.	0	+++	0	404.	0	+++	0
366.	0	+++	0	405.	0	+++	+++

Note: +++ all elements agglutinated
++ most of the elements agglutinated
+ the minority of the elements agglutinated
(+) only few of the elements agglutinated
0 no agglutination.

Excluding the spontaneously agglutinable strains, it will be seen that with three exceptions only — Nos. 245, 254, and 268 — all the strains examined with this technique were agglutinated by the non-absorbed serum, whilst the majority of the strains were not agglutinated by the serum that had been exhausted of agglutinin A. But a smaller number of the strains were agglutinable with serum 94/8 i. e. serum exhausted of the agglutinin for the antigenic factor A. Thus,

in these strains the agglutinin for the antigenic factor B alone had been able to cause agglutination.

This experiment does not show whether these strains contain all elements of the factor B as a common factor, or only contain part of it. In order to show this, absorption of serum 94 must be carried out with the strains; only the strains which completely exhaust serum 94/8 of agglutinins for strain 94 may be considered to contain all the elements of the factor B. This question has not been investigated.

Table 43.

List of the strains which showed agglutination on a microscopic slide with serum 94 after it had been absorbed with strain No. 8.

9—10—23—25—47—62—94—101—103—110—113—114—(116)—117—118—144—146—150—151—152—158—161—165—173—174—176—177—179—181—185—(187)—190—194—(208)—258—405—410=23—411=25.

Table 43 gives the strains that were strongly agglutinable with serum 94/8. It is seen that this group includes strains that have been isolated at places geographically far apart: the strains 9 and 10 were isolated in Siam, the strains 23 and 25 is Indochina, 94 (= 103) at the Sinai peninsula, most of the following strains in Northern China, and lastly, strain 405, at Shanghai.

In regard to the factor B, the question arises as to whether it is constantly present in these strains. It might very well be a labile factor, readily lost and readily appearing. In order to get some answer to this question, the following investigation was carried out:

In April 1934 fresh cultures of the strains 23 and 25, which had been included in the collection of the Institute in 1932, were received from l'Institut Pasteur at Saïgon. The culture corresponding to 23 was called 410 and the one corresponding to 25, 411.

Examination of these four cultures, which for 2 years had been kept and subcultured under different conditions, showed the presence of both factor A and B in all of them.

Further, the reactions of the cultures Nos. 94 and 103 show the same phenomenon, as they really represent the same strain. No. 94

was received at the Institute from N. C. T. C. in London; No. 103 was sent to the Institute by Professor van Loghem of Amsterdam. Here, also, we are dealing with strains which have been kept for some time under presumably differing conditions.

The above determinations of two factors, A and B, in the vibrios agglutinable with cholera serum agree quite well with the experimental results published by Scholten (1933). At present nothing can be said regarding possible correlation between these factors and the clinical picture of the disease produced by the vibrio; but this observation may be of importance in two ways, both in regard to the epidemiological examinations, and the choice of strains to be used for the production of vaccine.

Chapter VII.

DISCUSSION

In the introduction it was stated that the aim of the present investigations was to try to elucidate whether it was possible at present to produce an agglutinating standard cholera serum for use in general diagnostic work at the quarantine stations, amongst other places. Such a standard serum must agglutinate all the vibrios which cause clinical cholera, and it must agglutinate these vibrios only.

The answer to this question is that at present it is not possible to produce such an absolutely "faultless" agglutinating cholera serum.

Various points need further investigation.

One of the most outstanding problems is whether vibrios other than the cholera vibrio described by Koch, the classical V. Choleræ, can produce clinical cholera, as in the case of paratyphoid, for instance, where different well-characterized bacteria can cause the same clinical picture; in other words, the question is whether we must assume that within the group of the vibrios also there are several biologically and serologically characteristic, pathogenic types.

As a basis for investigating the pathogenicity of types other than the V. Choleræ, it is important, if not necessary, to possess a definite scheme according to which the vibrios may be grouped; this scheme should, if possible, be based upon several methods of investigation.

Hitherto the only basis for grouping used in general work has been the agglutination reactions, but in the present investigation it has been proved that not only is it possible, but also practical, to group the vibrios according to biochemical reactions, using their reactions towards mannose, saccharose and arabinose as a basis for the grouping.

The material examined here — about 400 strains of vibrios from numerous countries — may be divided into 6 types according to their reaction towards these 3 substances; this division is supported by the agglutination reactions, as different elements of the serological groups are mostly found within the same fermentation type.

In particular is worth noting the conformity of the fermentation reactions of the strains agglutinable with the cholera serum used. With one single exception — the V. Dunbar — every one of these 245 strains ferments according to the same type — called type I; i. e., they form acid with mannose and saccharose, but not with arabinose. Many of these vibrios were well-known cholera strains, and this serological group must be said to represent the typical cholera vibrio; moreover, this group included some of the much debated El Tor strains. Further, absorption experiments did not make it possible to distinguish between these vibrios and vibrios isolated from cases of clinically definite cholera.

Serologically there are two sub-groups within this group, characterized by the strains containing either the antigen group called A alone or together with the antigen group B. This fermentation type also included some smaller groups of serologically connected strains, one of which contained the Japanese type varié, another an El Tor strain.

Another fermentation type, type II — differing from type I in not fermenting mannose — includes groups with as many as 15 strains; a single group often includes strains that have been isolated at places far apart: Japan, China, Northern India, Egypt and other places.

The fermentation types that have been called III—VI only include comparatively few strains, most of which have not shown common serological characteristics.

In the present work it is quite impossible to say whether any of the strains that are non-agglutinable with cholera serum really have appeared as pathogenic microbes or whether they only represent incidental findings, consisting of non-pathogenic microbes from the patient's fæces. Information sent to us about some of the patients from whom vibrios included in the present material were isolated, shows that the mortality rate of the patients from whom non-agglutinable vibrios have been isolated is no smaller than that

of the patients who excreted cholera vibrios. It is possible only to determine whether a strain is pathogenic at the place where it has been isolated and where further examinations may be carried out, e. g., repeated examinations of fæces, examinations of the patient's blood for antibodies, examinations of the fæces of the individuals in the patient's environment, epidemiological examinations, etc..

Some of the Japanese investigations quoted make it seem probable that at Shanghai e. g. numerous cases of cholera have occurred, produced by a vibrio different to the typical cholera vibrio; and other examinations seem to indicate that in other places also there have been cases which clinically could not be distinguished from cholera, but in which cholera-like vibrios seemed to be the pathogenic microbes. Thus it seems that, if more of these microbes are found, it will become necessary to use several sera in bacterial diagnosis.

Further, a close examination of the thermolabile and thermostable antigens is necessary, partly for their presence as a common factor which may define certain groups, partly for the presence of specific or non-specific phases within the common factors. The questions of variation and the rapid appearance of rough forms are connected with the above problems.

Until it has been proved that strains which have been subcultured repeatedly and kept for long periods have not changed in any manner as regard their agglutinogenic or agglutinative powers, these examinations should be carried out at laboratories which have access to quite freshly isolated strains.

One other thing is of the greatest importance in work with a standard serum in diagnosis, i. e., an accurate definition of the technique to be used for the agglutination reactions. The importance of these is also shown in the present work.

Even though it appears that we are still fairly distant from our final aim, the production of an agglutinating serum to be used in general diagnosis, certain factors have been partly elucidated and partly reemphasized in the present work.

The most important point seems to be the denomination of certain fermentation groups in the vibrios; it seems justifiable to

exclude a vibrio as a typical cholera vibrio, basing oneself on the fermentation reaction with three carbohydrates alone, and when it is a question of whether or not a cholera vibrio is appearing in a non-agglutinable "phase", these reactions may possibly exclude such a possibility.

Serologically two groups have been defined in the vibrios agglutinable with cholera serum; they may be differentiated according to their content of the antigen groups A or AB; these findings agree with those of Scholten, but are independent of his investigations; further it has been found that in a material of about 400 vibrio strains the factor B has only been found in connection with A.

It has already been mentioned that different experiments have shown the importance of the agglutination technique in the results of the reactions; thus it has been found that whilst an agar culture emulsion of a strain may be non-agglutinable with a certain serum, formolized bouillon culture of the same strain may be quite agglutinable with the serum.

To this may be added the examination of the properties of thermolabile and thermostable antigens, compared with the corresponding reactions within the salmonella group. It was found that agglutination between thermolabile antigen and the corresponding agglutinin takes place comparatively slowly, and that the flocculation need not take place in emulsions of live culture, but that it takes place in formolized bouillon culture.

Agglutination between thermostable antigen and the corresponding agglutinin takes place comparatively quickly and it occurs in emulsions of agar culture as well as in bouillon cultures.

Agglutinin corresponding to the thermolabile antigen does not cause microscopically visible, immediate agglutination, nor does it paralyse the motility of the vibrios; on the other hand, agglutinin corresponding to the thermostable antigen causes immediate cessation of motility and microscopically visible agglutination.

Concerning the hæmolytic properties, it was found that power of hæmolysis may be a rather unstable property, at any rate in laboratory strains. It is mentioned, that according to the literature, cholera vibrios immediately after their isolation have in no case with a really reliable technique shown power of hæmolysis.

The old experience, that the cholera-red reaction may be caused by vibrios other than cholera vibrios was confirmed; there were only two cholera strains that did not produce the reaction.

In spite of the fact that several of the strains examined had been kept in laboratories for 10—20 years without animal passage, comma-shaped bodies were found in the majority of the strains. Only 23 of them were rod-shaped; one of these strains turned out to be a B. coli, but it seems possible that the other strains had previously been comma-shaped.

Abstract in English.

In 1932 the Cholera Commision appointed by the "Office International d'Hygiène Publique" asked Dr. Th. Madsen, amongst others, to arrange an investigation on the possibilities of producing an agglutinating standard cholera serum which might be used for practical diagnostic work. The work was prompted by the fact that at the quarantine station El Tor vibrios had been found which were agglutinable with the common cholera sera, and on closer examination these vibrios had turned out to be hæmolytic, a property which different quarters maintained was not found in true cholera vibrios. Further, epidemiological circumstances rendered it very unlikely that the vibrios in question were true cholera vibrios.

Dr. Madsen encouraged the author to undertake this investigation within the limits believed reasonable at a place where only laboratory examinations were possible, and where clinical and epidemiological aspects could not be considered in any degree of importance.

In the first few years after Robert Koch (1883—1884) had demonstrated V. Choleræ in the intestinal contents of cholera patients, the bacterial diagnosis was based upon morphological and cultural characters alone. After the serological reactions had been introduced in bacteriological diagnosis, the agglutination reactions became the decisive criterion of the bacterial diagnosis. A few attempts to introduce biochemical reactions did not lead to practical results. Amongst the vibrios which might be considered in the differential diagnosis there were only two groups: the V. Choleræ and the cholera-like vibrios, the latter group including the remainder of the vibrios. A few slight attempts have been made at defining pathogenic groups within the latter group, these attempts being mostly based on agglutinogenic properties.

Amongst the vibrios agglutinable with cholera serum some authors distinguish two groups, a large group including the most frequent non-hæmolytic strains, and a smaller group, including those

causing hæmolysis; it has not been possible to delimit these two groups by means of their antigenic composition or biochemical reactions.

The question of the presence and importance of O- and H-antigens within the cholera vibrios has so far not been definitely ascertained.

The material examined here consists of 384 strains collected more or less at random, as they have been sent from most of the districts where cholera and cholera-like diseases occur epidemiologically.

The power of acid formation of more than 100 of these strains was examined in 20 different substances: glucose, lævulose, galactose, mannose, lactose, saccharose, maltose, dextrine, glycogen, starch, arabinose, xylose, rhamnose, glycerine, adonite, mannite, dulcite, sorbite, salicin and tartrate. Only three of these substances were found to be of practical importance as regards the differential diagnosis: mannose, saccharose and arabinose.

Based on the reactions of the vibrios towards these three carbohydrates, 6 fermentation types were denominated:

Type I:	+ Mn,	+ Sa,	0 Ar.
Type II:	0 Mn,	+ Sa,	0 Ar.
Type III:	+ Mn,	+ Sa,	+ Ar.
Type IV:	0 Mn,	+ Sa,	+ Ar.
Type V:	+ Mn,	0 Sa,	0 Ar.
Type VI:	0 Mn,	0 Sa,	0 Ar.

+ indicating acid formation, 0 no acid formation, using ½ % concentration in peptone-water of the substance to be examined, and bromthymolblue as indicator.

Agglutination of the strains with a cholera serum showed that with one single exception all the strains agglutinable with the serum fermented like type I. Numerous strains, isolated from cases of clinical cholera, but non-agglutinable with the cholera serum, were found to ferment like type II; a few of the strains that were non-agglutinable with the cholera serum fermented like the types I, III, IV, V or VI.

It is possible to divide the vibrios non-agglutinable with the cholera serum into smaller groups according to their agglutinations;

these smaller groups mostly include strains of the same fermentation type. Strains isolated from cases of cholera-like diseases at places geographically far apart are often found within the same small group. It is not possible to say whether we are dealing with pathogenic microbes or not.

Preliminary examinations were made on the hæmolytic powers of bouillon cultures of different age upon the erythrocytes of several animal species: sheep, goat, horse, rabbit, man. On the basis of these examinations it was decided to examine the rest of the material for hæmolysis using 3 days' old bouillon cultures and goat's erythrocytes; but with the technique used here such great variations were found in the power of hæmolysis, that even bouillon cultures which had been made from colonies on the same agar plate sometimes showed opposite results. This experience caused the author to abandon hæmolysis as a basis for classification of the vibrios.

It was not possible by absorption experiments to find agglutinogens which made it possible to distinguish amongst the strains agglutinable with cholera serum the hæmolytic from the non-hæmolytic strains.

Attempts were made to find special groups within the vibrios agglutinable with cholera serum by the help of absorption reactions, and it was found that two groups could be distinguished — one in which the strains contained the antigenic factor A, and a smaller group containing two factors, called AB. The factor B was never found except in connection with factor A.

The finding of two factors is of importance in connection with epidemiological examinations and production of vaccine.

Examination of the differentiating influence of heat upon the antigens proved the existence of both thermostable and thermolabile antigens.

Agglutination using live agar culture emulsified in saline for antigen only gives agglutination with thermostable antigen. If formolized bouillon culture is used as antigen, agglutination with thermolabile antigen is also seen, appearing later than the agglutination with thermostable antigen.

Emulsion in serum of an agar culture killed by heating for a short time at 55 ° C. causes absorption between the thermolabile antigen and its corresponding agglutinin.

The agglutinin corresponding to the thermostable antigen is comparatively thermostable; the agglutinin corresponding to thermolabile antigen is thermolabile.

Microscopical examination in hanging drop preparations of the reaction between a serum containing agglutinin corresponding to the thermolabile factor only and a culture emulsified in this serum, did not at any point show agglutination or any influence upon the motility.

Thus, the thermolabile and thermostable antigens of the vibrios do not seem to be quite analogous to those of the salmonella group.

At the present time it does not seem possible to produce an agglutinating standard serum, if by this we understand a serum, which agglutinates all cholera vibrios and cholera vibrios alone. Before we can do this several problems must be investigated, e. g. whether any of the vibrios that have previously been termed cholera-like can appear as pathogenic, and if this is the case, which of them may appear in this manner and produce the clinical picture called asiatic cholera.

Further, there is the question of the presence of thermostable and thermolabile antigen as common antigens or group-dividing antigens among the vibrios.

These two problems may best be examined in districts where cholera is found, the first on account of the need for clinical, serological and epidemiological investigations, the second because the investigations in question should preferably be carried out with freshly isolated strains.

Anyone wishing to carry out clinical and epidemiological investigations in connection with bacteriological findings must necessarily be able to classify his material according to a definite scheme. The results of the present investigations offer a suggestion for such a classification; in this classification biochemical reactions play a fairly large rôle, contrary to what has previously been the case. The author is also of opinion that the biochemical scheme may be of help in deciding whether or not a vibrio non-agglutinable with cholera serum may be a cholera vibrio in a non-agglutinable phase or merely a cholera-like vibrio.

Dansk Resumé.

I 1932 opfordrede den af »Office International d'Hygiène Publique« nedsatte Kolerakommission Dr. med. Th. Madsen og andre til at lade undersøge Mulighederne for Fremstilling af et til praktisk diagnostisk Brug anvendeligt agglutinerende Standard Koleraserum. Den direkte Aarsag til, at denne Opgave blev taget op, var, at der gentagne Gange ved Karantænestationen El Tor var paavist Vibrioner, der var agglutinable med de almindeligt anvendte agglutinerende Kolerasera; men ved nærmere Undersøgelse viste disse Vibrioner sig hæmolytiske, en Egenskab, man fra flere Sider hævdede, ikke fandtes hos ægte Koleravibrioner. Hertil kom, at de epidemiologiske Forhold gjorde det usandsynligt, at det skulde dreje sig om ægte Koleravibrioner.

Paa Dr. Madsens Opfordring gik Forfatteren af nærværende Arbejde i Gang med Opgaven inden for de Rammer, som man fandt det rimeligt paa et Sted, hvor det kun var muligt at udføre Laboratorieundersøgelser uden at tage væsentlige Hensyn til Opgavens kliniske og epidemiologiske Sider.

I de første Aar efter at Robert Koch 1883—1884 havde paavist V. Cholerae i Tarmindhold fra Kolerapatienter, hvilede den bakteriologiske Diagnose af V. Cholerae alene paa de morfologiske og kulturelle Forhold. Efter Indførelse af serologiske Reaktioner i den bakteriologiske Diagnostik blev Agglutinationsreaktionen det afgørende Kriterium for den bakteriologiske Diagnose. Enkelte Forsøg paa at indføre biochemiske Reaktioner gav ikke noget praktisk Resultat. Blandt de Vibrioner, der differentialdiagnostisk kunde komme i Betragtning, arbejdede man i det væsentlige kun med to Grupper, nemlig V. Cholerae og de koleralignende Vibrioner, omfattende alle de øvrige Vibrioner. Enkelte mindre Forsøg har været gjort paa blandt disse sidste at afgrænse patogene Grupper, idet man ved

Grupperingen hovedsagelig har støttet sig til de agglutinogene Egenskaber.

Blandt de med Koleraserum agglutinable Vibrioner skelner nogle Forfattere mellem to Grupper, nemlig den hyppigst paaviste Gruppe, omfattende dem, der ikke er hæmolytiske, og en mindre Gruppe bestaaende af dem, der udviser Hæmolyse; en Adskillelse mellem disse to Grupper paa Grundlag af den antigene Sammensætning eller biochemiske Forhold har ikke været mulig.

Spørgsmaalet om O- og H-Antigeners Forekomst og Betydning inden for Koleravibrionerne er endnu ikke afklaret.

Det her undersøgte Materiale bestaar af 384 ret vilkaarligt indsamlede Vibrionstammer, tilsendt fra de fleste af de Egne, hvor Kolera og koleralignende Tilfælde optræder epidemisk.

Ved Undersøgelse af den syredannende Evne hos godt 100 af disse Stammer over for 20 forskellige Stoffer — nemlig Glukose, Lævulose, Galactose, Mannose, Lactose, Saccharose, Maltose, Dextrin, Glycogen, Stivelse, Arabinose, Xylose, Rhamnose, Glycerin, Adonit, Mannit, Dulcit, Sorbit, Salicin og Tartrat — syntes kun tre af praktisk differentialdiagnostisk Betydning; disse var Mannose, Saccharose og Arabinose.

Paa Grundlag af Forholdene overfor disse tre Sukkerarter opstilledes 6 Forgæringstyper, nemlig:

Type I:	+ Mn,	+ Sa,	0 Ar.
Type II:	0 Mn,	+ Sa,	0 Ar.
Type III:	+ Mn,	+ Sa,	+ Ar.
Type IV:	0 Mn,	+ Sa,	+ Ar.
Type V:	+ Mn,	0 Sa,	0 Ar.
Type VI:	0 Mn,	0 Sa,	0 Ar.

idet + betyder Syredannelse, 0 ingen Syredannelse ved Undersøgelse i en Koncentration paa ½ % af det undersøgte Stof i Peptonvand med Bromtymolblaat som Indicator.

Ved Agglutination af Stammerne med et Koleraserum fandtes samtlige hermed agglutinable Stammer, paa nær en enkelt, at forgære som Type I. Talrige Stammer, isolerede fra Tilfælde af klinisk Kolera, men inagglutinable med Koleraserum, viste sig at forgære som Type II; enkelte af de med Koleraserum inagglutinable Stammer forgærede som Type I, Type III, IV, V eller VI.

De med Koleraserum inagglutinable Vibrioner lader sig paa Grundlag af Agglutination inddele i talrige mindre Grupper, der for Størstedelen hver for sig rummer Stammer af samme Forgæringstype. Indenfor disse enkelte Grupper findes ofte Stammer, isoleret fra koleralignende Tilfælde fra vidt forskellige Egne. Hvorvidt det drejer sig om patogene Mikrober, er det ikke muligt at sige.

Paa Grundlag af orienterende Undersøgelser over Hæmolyse af Blodlegemer fra flere forskellige Dyrearter — Faar, Ged, Hest, Kanin, Menneske — med Bouillonkultur af forskellig Alder valgtes det at undersøge Materialet for Hæmolyse med tre Døgn gamle Bouillonkulturer og Gedeblodlegemer; men ved disse Undersøgelser fandtes, ihvertfald med den anvendte Teknik, den hæmolyserende Evne saa variabel, at selv Bouillonkulturer, anlagt fra Kolonier paa samme Agarplade, tilsaaet med samme Stamme, viste stik modsatte Forhold. Udfra denne Erfaring opgav man at benytte Hæmolyse som Grundlag ved Grupperingen.

Blandt de med Koleraserum agglutinable Stammer lykkedes det ved Absorptionsforsøg ikke at paavise Agglutinogener, der kunde adskille hæmolytiske fra ikke hæmolytiske Stammer.

Forsøg paa gennem Absorption at paavise særlige Grupper blandt de med Koleraserum agglutinable Vibrioner viste, at det indenfor disse var muligt at skelne mellem een Gruppe, indeholdende en Antigenfaktor kaldet A, og en anden mindre Gruppe, indeholdende to Faktorer, kaldet AB.

I intet Tilfælde er Faktor B paavist uden i Forbindelse med Faktor A.

Dette Forhold er af Betydning baade for epidemiologiske Undersøgelser og for Fremstilling af Vaccine.

Ved Undersøgelse over Varmepaavirkningens differentierende Indflydelse paa Antigenerne fandtes baade termostabile og termolabile Antigener.

Ved Agglutination med levende Agarkultur, opslemmet i Saltvand som Antigen, indtræder kun Agglutination med termostabilt Antigen. Ved Anvendelse af formaliniseret Bouillonkultur som Antigen iagttages tillige en senere indtrædende Agglutination af termolabilt Antigen.

Ved Opslemning af Agarkultur, dræbt ved kort Tids Opvarmning til 55 °, i Serum finder Absorption Sted mellem termolabilt Antigen og det tilsvarende Agglutinin.

Det til termostabilt Antigen svarende Agglutinin er relativt termostabilt; det til termolabilt Antigen svarende Agglutinin er termolabilt.

Ved mikroskopisk Undersøgelse i hængende Draabe af Reaktionen mellem et Serum, der kun indeholder Agglutinin svarende til den termolabile Faktor, og en deri opslemmet Kultur iagttoges ikke nogen øjeblikkelig indtrædende Agglutination eller Paavirkning af Bevægeligheden.

De termolabile og termostabile Antigeners Forhold hos Vibrionerne synes saaledes ikke at danne en fuldstændig Parallel til de tilsvarende Forhold hos Salmonellagruppen.

Det synes ikke muligt paa det nuværende Tidspunkt at fremstille et agglutinerende Standard Koleraserum, hvorved maa forstaas et Serum, der agglutinerer alle Koleravibrioner og kun disse. Flere Problemer trænger forinden til nærmere Opklaring, f. Eks. Spørgsmaalet om, hvorvidt nogle og da hvilke af de som koleralignende Vibrioner tidligere benævnte Stamme, der kan optræde som patogene Mikrober og foraarsage det kliniske Billede, der betegnes Cholera asiatica. Endvidere Spørgsmaalet om de termolabile og termostabile Antigeners Forekomst som fælles Antigener og gruppeafdelende Antigener indenfor Vibrionerne.

Begge disse Spørgsmaal løses bedst paa de Steder, hvor Kolera findes, det første af Hensyn til de nødvendige kliniske, serologiske og epidemiologiske Studier, det andet fordi disse Undersøgelser bedst udføres med frisk isolerede Stammer.

For den, der vil arbejde med kliniske og epidemiologiske Undersøgelser i Tilslutning til det bakteriologiske Fund, er det nødvendigt at kunne gruppere sit Materiale efter et bestemt System. Resultatet af nærværende Arbejde er et Forslag til en saadan Gruppering, hvor i Modsætning til tidligere Forsøg de biochemiske Undersøgelser tillægges en ikke ringe Betydning. Forfatteren mener ligeledes, at det biochemiske System kan yde en Hjælp til Afgørelse af, hvorvidt én med Koleraserum inagglutinabel Vibrion kan være en Koleravibrion i inagglutinabel Fase, eller om den er en koleralignende Vibrion.

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